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JOURNAL OF FOOD SCIENCE AND TECHNOLOGY

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Research Papers

Research Notes

Fermentative Production of Citric Acid by *Aspergillus niger*: Strain Selection and Optimum Cultural Conditions for Improved Citric Acid Production

A. K. BANIK
Department of Food Technology and Biochemical Engineering, Jadavpur University, Calcutta-700 032

Manuscript Received: 9 *May* 1974

In the course of mutation studies of *Aspergillus niger* strain AB with ethylene imine (1:4000), a mutant *A. niger* AB 501 produced greater amount of citric acid in the culture broth (44.5 mg/ml) as against the parent strain (11.6 mg/ml) by the surface culture method of fermentation. This mutant was then exposed to UV-rays and a mutant, *A. niger* AB 1801, was found to produce high citric acid in the culture broth (60.0 mg/ml). Sucrose at a level of 15% gave the maximum yield of citric acid (70.5 mg/ml). The optimum concentration of ammonium nitrate was 2.2 mg/ml. The optimum cultural conditions for the production of citric acid by *A. niger* AB 1801 are: pH, 3.5; temperature, 27°C; incubation period, 9 days. The maximum yield of citric acid in the above conditions was 80.2 mg/ml. Addition of sodium monofluoroacetate (50 mg/lit) to the fermentation medium increased the citric acid production (120.4 mg/ml) whereas, addition of potassium ferrocyanide considerably decreased the citric acid production.

A number of organic acids are produced by molds and bacteria through the process of fermentation, and citric acid is one of the most important metabolic products now produced commercially by fermentation with specific molds, mostly strains of *Aspergillus niger*. Wehmer[1] was the first to report the citric acid produced from sugar through fermentation by molds, named by him as *Citromyces pfefferians* and *C. glaber*. Later, many other fungi have been found to ferment sugar to produce citric acid, but today some strains of *A. niger* are used for commercial production of citric acid in many countries. Mutagenic improvement of industrial micro-organisms has become a common practice[2]. High citric acid-yielding mutants can be isolated from *A. niger*[3-5], but studies are required to find how the induced mutants behave under varied cultural conditions. The author carried out a series of mutagenic treatments with *A. niger*, with a view to improve the yield of citric acid. The optimum concentration of sucrose and ammonium nitrate and the effects of pH, time of fermentation, temperature, some inhibitors to improve the yield of citric acid by the mutant, *A. niger* AB 1801, are also reported in this paper.

Materials and Methods

Development of the mutant strains of A. niger: In the course of mutation studies with ethylene imine and ultraviolet rays of *A. niger* strain about 1,976 mutant strains were isolated. Out of these mutants only *A. niger* AB 1801 which produced 11.6 mg/ml citric acid, was selected for further studies.

Medium and cultural conditions: The parent culture, *A. niger* strain AB, and mutant strains were maintained on malt-extract and yeast-extract agar slants at 4°C. The Czapek-Dox agar medium was used for the mutation studies. The medium I was used for the fermentative production of citric acid consisting of sucrose, 12; NH_4NO_3, 0.22; $MgSO_4.7H_2O$, 0.02; K_2HPO_4, 0.1 per cent and pH, 3.5. When the cultures were needed for mutation studies, they were transferred to slants of malt extract and yeast extract agar and incubated at 27°C for 7 days for sufficient sporulation. Spore crops were harvested by washing the slant with sterile distilled water and filtering the resulting spore suspension through several layers of sterile absorbent cotton. The spore density was adjusted to 2.6×10^7 per ml of the suspension. This spore suspension was used both for mutation studies and for the inoculation of the fermentation medium. Surface culture fermentation was carried out using 500 ml flat flasks, each containing 150 ml of medium. The flasks were then incubated at 27°–28°C for 8 days.

Various factors influencing the production of citric acid: The optimum cultural conditions for the production of citric acid by *A. niger* AB 1801 were worked out by keeping all the factors constant except the one which was varied. The factors studied were (*a*) different concentrations of sucrose, (*b*) different concentrations of NH_4NO_3, (*c*) pH of the medium, (*d*) time period of fermentation, and (*e*) temperature of fermentation. Fermentation conditions were the same as described before.

Determination of citric acid concentration: Aliquots

of fermented medium were removed periodically and the total acidity in each was determined by titration with 0.1 N NaOH using methyl red as indicator. On the days of peak acid production, the contents of each flask were filtered and citric acid in the filtrate was estimated by converting to pentabromoacetone, according to the method described by Snell and Snell[6]. The residual sugar was determined by Summer's[7] 3,5-dinitrosalicylic acid method.

Results and Discussion

Selection of mutants of A. niger: The parent culture used in the experiment was *A. niger* strain AB, selected out of hundred isolates of the fungus from various sources. Spore suspension of this parent strain, containing 2.6×10^7 per ml, was then treated with ethylene imine and UV-rays. In the ethylene imine treatment, it has been observed that a concentration of 1:4000, of the mutagen is most effective for mutation. This concentration was, therefore, used for the treatment of spores of the parent cultures. After treatmen. for 1,2,3,4, and 5 hr the spore suspension in each case was diluted and plated out in Czapek-Dox (CD) agar medium. 1,256 isolates were selected from different stages of treatment with ethylene imine for the citric acid production. It was observed that the mutant, *A. niger* AB 501, gave higher yield of citric acid (44.5 mg/ml) in medium I. The spores of the mutant, *A. niger* AB 501 were then exposed to UV-rays at the distance of 12 cm. The source of radiation was a Hanovia germicidal lamp (15 Watt). Samples were irradiated for 10,15,20,25 and 30 min. The treated spores were plated out in Czapek-Dox agar medium. Colonies were then transferred to malt-extract and yeast-extract agar slants. 720 isolates were selected, from different stages of treatment with UV-rays, for citric acid production. It was observed that the mutant *A. niger* AB 1801 gave the highest yield of citric acid (60.0 mg/ml) in medium I. The most promising mutant, *A. niger* AB 1801, was therefore, studied to standardize the cultural conditons for fermentation.

Effect of different concentration of sucrose: It has been found that sucrose is the best carbon source for the citric acid production. Table 1 indicates that the optimum level of sucrose in the medium I for the production of citric acid is 15 per cent.

Effect of different concentration of ammonium nitrate: Different nitrogen sources such as urea, ammonium sulphate, ammonium nitrate, ammonium chloride, sodium nitrate, ammonium acetate, ammonium tartrate, ammonium oxalate, diammonium hydrogen phosphate were then examined for their effect on citric acid production. It has been found that ammonium nitrate is the superior nitrogen source for the citric acid production. Table 2 shows that the optimum level of ammonium nitrate for the citric acid production was 0.22 per cent.

Effect of pH on the citric acid production: The initial pH of the medium was adjusted to 2.0, 2.5, 3.0, 3.5, 4.0, 4.5 and 5.0 with 1N HCl or NaOH. Fig. 1 shows that the optimum pH for the citric acid production is 3.5.

TABLE 1. EFFECT OF DIFFERENT CONCENTRATIONS OF SUCROSE ON CITRIC ACID PRODUCTION BY *Aspergillus niger* AB 1801

Sucrose supplied (mg/ml)	Ammonium nitrate supplied (%)	Sucrose utilized (mg/ml)	Total acid (ml N acid per 100 ml)	Citric acid* (mg/ml)	% Conversion
100	0.22	100	84.0	51.2	51.2
120	0.22	120	99.1	60.0	50.0
150	0.22	142	110.3	70.5	46.7
160	0.22	146	102.5	62.3	39.0

*Values are averages of triplicates.

TABLE 2. EFFECT OF DIFFERENT CONCENTRATIONS OF AMMONIUM NITRATE ON CITRIC ACID PRODUCTION BY *Aspergillus niger* AB 1801.

Ammonium nitrate (%)	Sucrose supplied (mg/ml)	Total acid (ml N acid per 100 ml)	C/N ratio	Citric acid (mg/ml)
0.10	150	40.3	191	21.2
0.15	150	78.6	129	48.0
0.20	150	102.2	96	62.2
0.22	150	110.3	87	70.5
0.25	150	108.4	76	70.2
0.30	150	104.0	64	70.0

*Values are averages of triplicates.

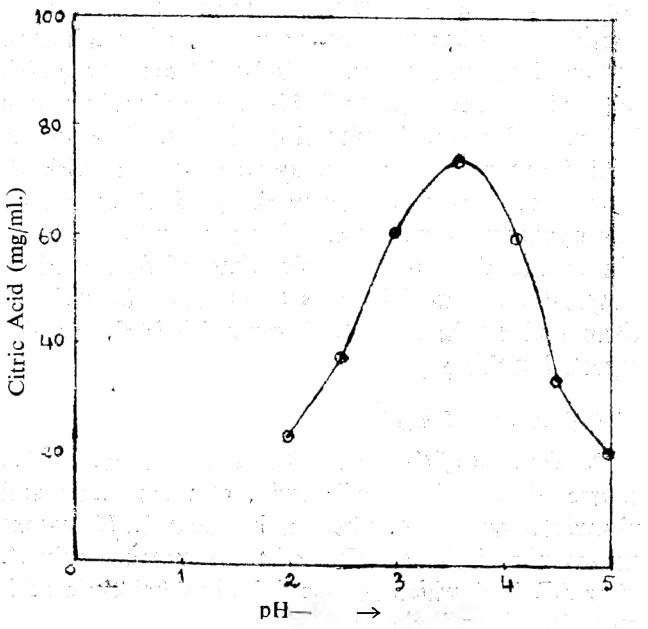

FIG 1. Effect of pH on the citric acid production by *Aspergillus niger* AB 1801.

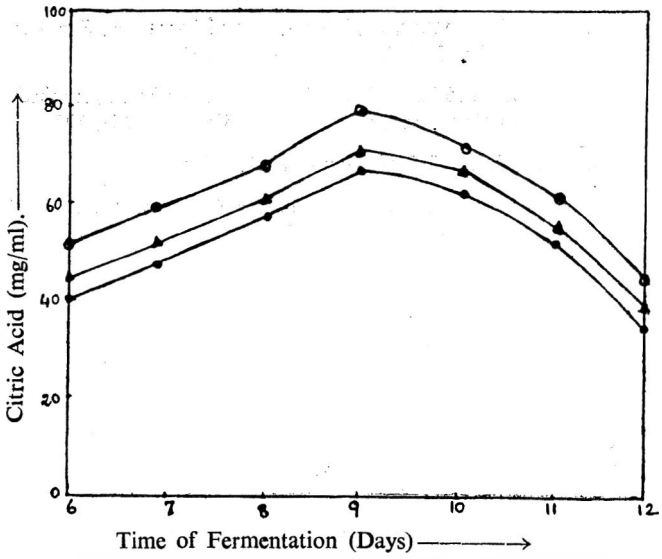

FIG 2. Effect of temperature and fermentation period (days) on the citric acid production by *Aspergillus niger* AB 1801.
0—0 27°C; △—△ 25°C; ●—● 30°C.

Effect of temperature and fermentation time on the citric acid production: The effect of different temperature and fermentation time on the citric acid production are shown in Fig. 2. The optimum period required for fermentation for citric acid production was 9 days. A temperature of 27°C optimally favoured the production of the citric acid (80.2 mg/ml) and a lower or a higher temperature significant by decreased the yield of the citric acid.

The final composition of the medium and cultural conditions for the citric acid production by *A. niger* AB 1801 were maintained as follows:

Sucrose, 15.0; NH_4NO_3, 0.22; K_2HPO_4, 0.1; $MgSO_4.7H_2O$, 0.02 per cent; pH, 3.5; fermentation time, 9 days and temperature, 27°C.

Effect of various substances on citric acid formation: Various known substances, having significant influence on citric acid production, were included at the start of the fermentation. The results are shown in Table 3. The addition of sodium monofluoroacetate increased the citric acid production. On the other hand, the addition of potassium ferrocyanide decreased the production of citric acid. No effect was observed from the addition of methyl alcohol and sodium succinate. The cumulative amount of citric acid was remarkably increased by the addition of an aconitase inhibitor such as sodium monofluoroacetate but decreased by the addition of potassium ferrocyanide because ICDH activities of the cells were lowered[8].

TABLE 3. EFFECT OF VARIOUS SUBSTANCES ON CITRIC ACID PRODUCTION BY *Aspergillus Niger* AB 1801

Compound added	Amount added (μ mole)	Citric acid (mg/ml)*
No addition	—	80.2
Sodium monofluoroacetate	50	95.0
Potassium ferrocyanide	150	20.8
Methyl alcohol	2000	80.0
Sodium succinate	150	80.2

*Values are averages of triplicates.

TABLE 4. EFFECT OF ADDITION OF SODIUM MONOFLUOROACETATE ON CITRIC ACID PRODUCTION BY *Aspergillus Niger* AB 1801

Sodium monofluoroacetate (mg/litre)	Time of addition (hr)	Citric acid (mg/ml)*
0	—	80.2
10	0	80.2
30	0	98.5
50	0	100.0
100	0	90.4
10	24	80.2
30	24	105.0
50	24	120.4
100	24	95.0

*Values are averages of triplicates.

Effect of different concentrations of sodium monofluoroacetate on citric acid production: The production of citric acid was increased by the addition of sodium monofluoroacetate. Table 4 indicates that the production of citric acid remarkably increased by the addition of 50 mg/litre of sodium monofluoroacetate after 24 hr of fermentation. It is also found from Table 4 that a lower concentration of sodium monofluoroacetate (10 mg/lit) had no effect on citric acid production when added to the medium both at 0 and 24 hr of fermentation but if the sodium monofluoroacetate concentration was further increased there was increased citric acid production which was much greater if the sodium monofluoroacetate was added after 24 hr of fermentation.

Further studies are in progress to test this culture to assess its industrial utilization for large scale production of citric acid.

Acknowledgement

Grateful acknowledgement is made to Prof. A. N. Bose, Vice-Chancellor of Jadavpur University, for his encouragement in this work. The author also wishes to thank Dr S. K. Majumdar, for his kind help in this work.

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ERRATA

Following corrections are to be made in the article indicated below:

(1) "Studies on Papads based on blends of black gram with cereals, pulses and starches" (2) "Brabender farinograph as a tool in the objective evaluation of papad dough" by S. R. Shrupalekar and K. V. L. Venkatesh published in this Journal 1975, **12** (1), 32 and 36.

Page	*Column*	*Para*	*Line*	*Printed as*	*Corrected as*
32	—	—	Title	Papada	Papads
33	2	3	6	Green gram other belnds	Green gram were 40 to 42% as compared to 45% for other blends
33	Table 1	—	Text 1st line	Creamy yellow yellow	Creamy yellow
30	2	4	5	Jeera flaskes, 20	Jeera flakes, 2.0
37	Fig. 2	—	Under foot note	Black gram: green gram	Black gram with: green gram
38	Table 1	—	2 foot note	which did contain	which did not contain.
39	1	2	10	and wheat was soft	and wheat it was
41	1	4	11	required eatra dusting	rrquired extra dusting
41	2	References	8	(Communicated)	1975, **12**, 32

Studies on Hydrogen Sulphide in Cheddar Cheese at different Stages of Its Manufacture and Ripening

C. M. KAPOOR, JOGINDER SINGH AND R. V. RAO*
National Dairy Research Institute, Karnal, Haryana, India

Manuscript Received: 27 *November* 1974

Samples of cow and buffalo milk cheeses at different stages of manufacture were studied. The raw milk of cow and buffalo had no hydrogen sulphide. After pasteurization the buffalo milk had slightly higher value as compared to cow milk. The curd at pitching stage had slightly higher hydrogen sulphide in buffalo milk. The green cheese of both cow and buffalo milk had almost the same value. A gradual increase in hydrogen sulphide was observed during ripening upto a period of 105 days, the cow milk cheese having higher value than the buffalo milk cheese.

Kristoffersen *et al.*[1] reported higher concentrations of hydrogen sulphide in cheddar cheese from raw cow milk as compared to the cheese from pasteurized cow milk and the same was responsible for perceptible differences in the flavours of these two cheeses. The presence of minute quantities of hydrogen sulphide and other sulphur compounds seems to be essential for a desirable aroma in cheddar cheese. An insufficient or excessive amount of these compounds beyond a certain level may be undesirable. Mabitt[2] propounded that metabolic changes of sulphur compounds in cheese which resulted in the production of hydrogen sulphide might serve as an indicator of flavour production. Kristoffersen and Gould[3–5] established a significant correlation between hydrogen sulphide and typical cheddar cheese flavours. Kristoffersen and Nelson[6] reported that after six months ripening, cheeses containing the highest relative concentration of free hydrogen sulphide received the highest cheddar cheese flavour intensity score. Walker[7] was unable to stimulate cheddar type flavour by the incorporation of ketones and fatty acids, unless thioacetamide, a source of hydrogen sulphide, was added.

The changes taking place in hydrogen sulphide at different stages of manufacture and ripening of cheddar cheese have however not been reported systamatically in the literature. Hence an attempt was made in the present study to follow up these changes in cheddar cheese from cow and buffalo milks.

Materials and Methods

Cheddar cheese was manufactured at the Experimental Dairy of NDRI, Karnal, from standardised (3.5 per cent fat and 9 per cent SNF) cow and buffalo milks, which were pasteurized by HTST method. In all eight batches of cheese, four each of buffalo and cow milks were prepared. A mixed starter culture consisting of *Streptococcus lactis* and *Streptococcus cremoris* was used. The ripening of cheese was carried out in the cold storage room under controlled conditions of 75 per cent relative humidity and at $10° \pm 0.5°C$.

Hydrogen sulphide was determined by the p-aminodimethylaniline (PAD) method of Badings and VanDer Pol[8] and of Kristoffersen *et al*[1] with some modifications. Alkaline pyrogallol washed air was used for aspiration. Hydrogen sulphide gets converted to methylene blue and colour read in a Beckman DU spectrophotometer, at 665 $m\mu$ using distilled water as a reference. The values were read against a standard curve.

Results and Discussion

Data on the hydrogen sulphide at different stages of manufacture is presented in Table 1 for cow and buffalo milk. It has been observed that raw cow and buffalo milks had no hydrogen sulphide. However, after pasteurization the buffalo milk showed a higher value of 14.79 as compared to 11.90 μg/100g of cow milk. When starter was added there was a decrease in the hydrogen sulphide in the milks of both cow and buffalo. There was a further decrease when rennet was added to both the milks apparently due to the influence of light, oxygen and volatilization of hydrogen sulphide at temperatures of renneting and time of holding. Badings and VanDer Pol[8] also reported a similar influence of light, oxygen and volatilization on the hydrogen sulphide.

The hydrogen sulphide of whey at pitching stage increased in cow and buffalo milks because of an increased heat treatment from cutting to pitching stage. The curd was observed to contain less hydrogen sulphide as compared to whey. This may be due to albumin and especially β-lactoglobulin, which is the primary source

*Present address: S.R.S., National Dairy Research Institute, Bangalore.

TABLE 1. HYDROGEN SULPHIDE (μG/100 G) AT VARIOUS STAGES OF MANUFACTURE OF COW AND BUFFALO MILK CHEDDAR CHEESE

Particulars	Values of hydrogen sulphide									
	Cow milk Trial No.					Buffalo milk Trial No.				
	I	II	III	IV	Average	I	II	III	IV	Average
Raw milk	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Pasteurised milk	11.54	11.54	12.98	11.54	11.90	12.98	12.98	15.87	17.31	14.79
Milk+starter	11.54	10.39	11.54	9.24	10.68	11.45	12.98	12.98	11.54	12.26
Milk+rennet	7.21	5.77	9.24	7.21	7.36	5.77	7.21	7.21	5.77	6.49
Whey at pitching	8.36	7.21	10.39	8.36	8.58	7.21	5.77	7.21	11.54	7.93
Curd at pitching	2.88	1.15	2.88	1.15	2.02	1.15	2.88	2.88	2.88	2.45
Whey at drainage	5.77	5.77	7.21	9.24	7.00	5.77	2.88	3.03	2.88	3.64
Green cheese (at hooping)	1.15	Nil	1.15	1.15	0.86	Nil	1.15	1.15	1.15	0.86

of sulphydryls, being released in whey[9]. The buffalo milk curd had a slightly higher hydrogen sulphide—2.45 μg/100g as compared to 2.20 μg/100g of cow milk curd at pitching stage. Whey at the time of drainage had a different picture, the buffalo milk whey having just half the concentration than that of cow milk whey. However, the values were more or less similar in the green cheese of both cow and buffalo milks. The quantities of hydrogen sulphide in green cheese were the lowest. This fact resembles the observations of Swiatak and Poznanski[10] who reported the retarding effect of common salt on hydrogen sulphide content in trappist cheese.

After waxing, the cheese blocks were kept for ripening. The cheese samples were analysed at fortnightly intervals for hydrogen sulphide. A gradual increase in hydrogen sulphide observed during 105 days ripening is depicted in Fig. 1. The concentration of hydrogen sulphide varied

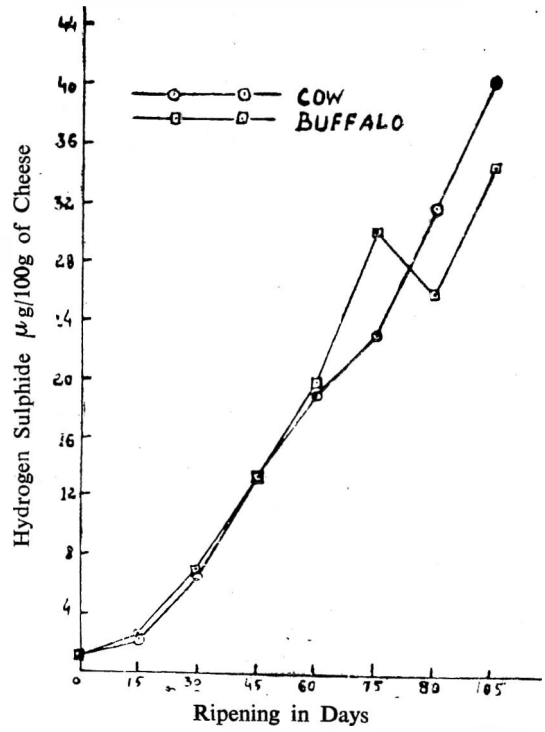

Fig. 1. Hydrogen sulphide content during ripening

from 0.86 to 34.62 μg per 100g in buffalo milk cheese and from 0.86 to 40.38 μg/100g in cow milk cheese. Lawrence[11] reported an average hydrogen sulphide concentration of 55 μg/100g of cheese, after 1 to 4 months of ripening in New Zealand cheddar cheese. Our present findings are in fair agreement with the above report. After 75 days of ripening, fluctuation in hydrogen sulphide was observed. Such type of fluctuations have also been reported by Kristoffersen and Gould[5]. After 105 days of ripening, cow milk cheese excelled in flavour and acceptability as compared to buffalo milk cheese. This clearly indicates an existence of a correlation between the cheese flavour and its hydrogen sulphide content. Grade 'A' cheddar cheese was always found to possess higher hydrogen sulphide concentration as reported by various workers[3–5] and this had been significantly correlated with their flavours. Walker[7] also established that the presence of hydrogen sulphide was necessary for an improved cheddar type flavour.

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Processing and Nutritional Evaluation of Calcium Caseinate

M. R. SRINIVASAN, M. V. L. RAO AND K. S. SRINIVASAN
Central Food Technological Research Institute, Mysore 570013, India

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Comparisons of the essential amino acid composition and protein efficiency ratio determined by rat bioassay have shown that the processing steps involved in the calcium-sucrate method of preparing calcium caseinate exert no deleterious effects on the essential amino acid makeup or physiological utilization. Use of ammonium hydroxide for dispersing and processing the protein appears to be best as it causes no loss of the phosphate groups required for binding the calcium and leaves no salt residues in the end product.

Calcium caseinate is a readily acceptable protein supplement which can be effectively employed in the clinical management of protein-calorie malnutrition and related disorders. It is easily dispersible in water and can be conveniently administered in regulated doses either in the form of a beverage by itself or added to other beverages or food preparations. A novel process[1,2] for the production of this valuable proteinate was developed at this institute based on the use of calcium sucrate for incorporating the calcium into the protein. The process comprises the following steps: (*a*) Washing the casein in hot (70°C) water, (*b*) dispersing it in ammonia, (*c*) adding the requisite quantity of calcium as calcium hydroxide in sucrose solution, and (*d*) finally roller drying (50 psi steam pressure, 130–140°C).

It is known that heat treatment under certain conditions is detrimental to the nutritional quality of protein mainly because some amino acids are destroyed and certain others rendered non-available[3]. The deleterious effect of heat on the nutritive value of milk proteins and particularly casein has been reported[4–6]. The effect appears to be proportional to temperature as well as time. The damage has been traced to decreased digestibility and consequent lowered growth promoting value for rats. A similar effect has also been observed in dogs[7]. A significant decrease in the protein efficiency ratio of casein subjected to dry heat over 100°C or to heating at 95°C in aqueous medium, as compared with raw casein (acid precipitated commercial casein) has been observed.

Sulphur amino acids as well as serine and threonine in peptide combination are known to be adversely affected by alkali treatment[8]. Alkali lability[9–12] is a characteristic feature of the phosphate groups in casein. On heating in sodium hydroxide solution, the phosphate groups are cleaved off readily and quantitatively through a process of β-elimination with the initial formation of dehydroalanine. As the protein bound phosphate groups, mostly present as o-phosphoserine residues, are the primary sites of calcium binding, the effect of alkalis on the splitting of phosphate is also an important factor to be reckoned in the processing of casein.

In view of these considerations, experiments were designed to ascertain the effect of the processing steps involved in the preparation of calcium caseinate, on the nutritive value of the product as compared with the original raw casein. The effect of alkalis on the cleavage of phosphate from the protein was also examined.

Materials and Methods

Casein: Fine grade, lactic casein from a commercial source was used for the preparation of the calcium caseinate.

All chemicals used for the analytical work were of ANALAR quality.

Proximate analysis: Standard procedures of the AOAC[13] were followed for the analysis of proximate principles. Calcium was analysed according to the method of Clark and Collip[14]. The proximate composition of the casein and calcium caseinate were:

	Casein	Calcium caseinate
Protein convertion factor	6.38	6.38
Moisture (%)	10.5	4.5
Protein (%)	83	84
Fat (bound) (%)	1.2	1.3
Sucrose (%)	nil	5.8
Phosphorus (%)	0.89	0.8
Calcium (%)	Traces	1.0

Amino acid composition: The essential amino acids content of the casein and of calcium caseinate prepared from it were determined according to microbiological procedures described by Barton-Wright[15]. The stored sample analysed had kept well for 24 months at room temperature (ambient temperature varying between 22°–32°C over the seasons) in air in sealed polythene bags.

Nutritive value: The PER of raw casein and calcium caseinate were assayed on weanling albino rats at 4 and 8 week periods by the method of Osborne, Mendel and Ferry[16]. The diet contained 10 per cent protein, 10 per cent fat (refined groundnut oil), 2 per cent salt mixture[17] and 1 per cent vitamin mixture[18]. At the end of the 8 week feeding period, blood was drawn by cordiac puncture for determination of serum proteins by the biuret procedure[19] and the animals were sacrificed and livers excised for the determination of total protein.

Alkali cleavage of phosphate: For studying the release of phosphate from casein by alkalis, 10 per cent (w/v) dispersions of the protein were prepared in 0.25, 0.5 and 1 N aqueous sodium hydroxide or ammonium hydroxide solutions and incubated at 37°C for 1,3,5,7 and 24 hr. The inorganic phosphate released was estimated in TCA (10%) filtrates of aliquots of the incubate.

TABLE 1. ESSENTIAL AMINO ACID COMPOSITION OF CALCIUM CASEINATE

Amino acid	Casein	Calcium caseinate	
		Fresh	After 2 yr storage at room temp
	g amino acid/16 g N		
Arginine	4.3	4.1	4.2
Histidine	2.0	1.8	1.9
Lysine	6.0	5.9	5.8
Tryptophane	1.3	1.4	1.3
Phenylalanine	4.9	5.0	4.9
Methionine	2.9	3.0	2.9
Threonine	4.7	4.7	4.9
Leucine	11.0	10.5	11.7
Isoleucine	7.2	7.1	6.7
Valine	7.5	7.4	7.4

All the amino acids were estimated by microbiological assay.

Results and Discussion

As is evident from the results in Table 1, there is very little difference in the essential amino acids makeup between the casein and the calcium caseinate derived from it according to the method developed[1]. Furthermore, even upon storage for two years, the amino acid composition of the calcium caseinate had shown little alterations. The findings support the conclusion that the processing steps and conditions adopted have neither caused destruction of the essential amino acids in the casein nor have they initiated any changes which on long storage over 24 months could slowly lead to their degradation.

Similarly, there is no difference in either intake, digestibility or PER between the two proteins (Table 2) nor in the serum and total liver protein levels between the animals fed on the two proteins (Table 3). The results are in accord with their amino acid composition and indicate that the processing steps followed in the preparation of the calcium caseinate have not impaired to any measurable extent the essential amino acids in the casein or their physiological availability and utilization.

The study of the release of inorganic phosphate from casein by alkalis (Figure 1) has demonstrated that sodium hydroxide at 0.25 N and ammonium hydroxide at concentrations between 0.25 and 1.0 N (pH 7) and at

TABLE 2. PROTEIN EFFICIENCY RATIO (PER) OF CASEIN AND CALCIUM CASEINATE

Diet	Four week period					Eight week period			
	Initial body wt (g)	Diet intake (g)	Protein intake (g)	Gain in body wt (g)	PER	Diet intake (g)	Protein intake (g)	Gain in body wt (g)	PER
	Males								
A. Casein	46.3	234.7	24.18	47.8	1.98	579.3	59.47	95.7	1.61
B. Calcium caseinate	46.2	249.8	25.74	58.3	2.27	587.7	60.52	108.5	1.80
Diff in PER (B-A) with standard error	—	—	—	—	0.29±0.19 NS*	—	—	—	0.19±0.14 NS*
	Females								
A. Casein	46.0	226.5	23.3	52.8	2.26	516.7	53.2	93.5	1.76
B. Calcium caseinate	45.7	258.2	26.6	61.2	2.31	609.7	62.9	113.0	1.79
Diff in PER (B-A) with standard error	—	—	—	—	0.05±0.09 NS*	—	—	—	0.03±0.07 NS*

The proteins were used at 10 per cent level in standard test diets.

Six male and 6 female rats were distributed into the two groups according to the randomised block design, figures represent average.

*NS: Not significant.

TABLE 3. SERUM PROTEIN AND LIVER PROTEIN LEVELS IN RATS FED CASEIN AND CALCIUM CASEINATE

Diet	Serum protein (g/100 ml)						Liver protein (total N) g/100g body wt	
	Albumin		Globulin		Total			
	M	F	M	F	M	F	M	F
A Casein	4.42	5.03	1.89	1.30	6.59	6.52	0.18	0.16
B Calcium caseinate	3.84	4.86	2.19	1.62	6.03	6.49	0.18	0.18
Standard error	0.16 (4 df)	0.16 (4 df)	0.43 (4 df)	0.21 (4 df)	0.34 (5 df)	0.20 (5 df)	0.011 (5 df)	0.183 (5 df)
Test of significance (A ~ B)	*	NS	NS	NS	NS	NS	NS	NS

Six male (M) and 6 female (F) rats were distributed into the two groups according to the randomised block design; figures represent average.

As one sample of serum in the male and one in the female rats was lost in the casein-fed group, the corresponding albumin and globulin values were estimated by the missing plot technique. The averages include these also.

NS: Not significant. *Significant at 5% level.

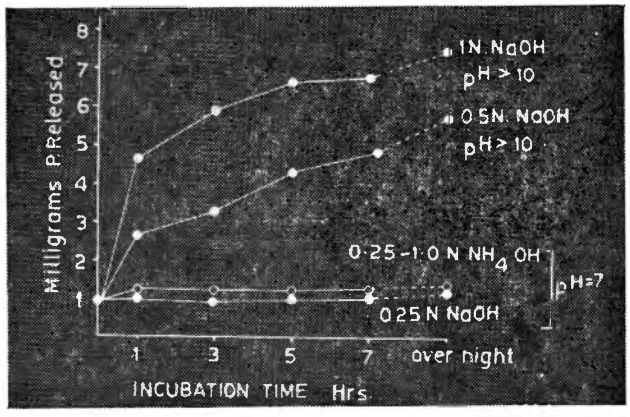

Fig. 1. Release of inorganic phosphate from casein by alkalis mg P_i released / g protein in 10 ml aqueous dispersion.

37°C do not split off phosphate to any measurable extent while sodium hydroxide at 0.5 and 1.0 N concentration (pH>10) rapidly hydrolyses the phosphate ester bonds. The accelerated release is obviously a pH effect. Ammonium hydroxide at 1 N concentration appears to be doubly innocuous because it does not cleave the serine (and threonine) bound phosphate groups of casein and evaporates off during the roller-drying without leaving any undesirable salt residues in the end product. Further, even at the high temperature attained during the roller drying no phosphate appears to be severed off from the casein dispersed in the ammonia-calcium sucrate medium as evidenced by the fact that the phosphate content of the casein and of calcium caseinate are almost the same. These observations are in conformity with those of other investigators[20] who have also employed ammonia hydrolysis for preparing the major phosphopeptides of casein in quantity.

Acknowledgement

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Enzymic Saccharification of Bagasse

S. N. MUKHOPADHYAY*, P. GHOSH** AND V. H. POTTY**
Biochemical Engineering and Food Technology Department, H.B.T.I., Kanpur, UP

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An attempt has been made to saccharify bagasse enzymatically. Cellulase in the culture filtrate from *Trichoderma viride* QM 6a caused saccharification. Optimum saccharification was found to take place at pH 5.1, temperature, 50°C and at a substrate concentration to culture filtrate ratio of 2 mg per ml of culture filtrate (having activity of 35 units per ml). Bagasse obtained by preliminary treatment when further modified by heating followed by grinding, alkali treatment and neutralization could saccharify bagasse to an appreciable extent of 24% based on the amount of bagasse used in reaction mixture. Cell propogation studies with liquid centrifugate of bagasse hydrolyzate indicated that it may serve as a substrate for food yeast propogation.

Cellulosic materials which occur abundantly in nature can be hydrolysed by cellulases to fermentable sugar[1–4]. Among the organisms strains of *Trichoderma viride* are now widely used to produce cellulase for cellulose saccharification. Enhancement of saccharification of cellulose by cellulase through proper modification of cellulosic substrate has been reported[5]. Bagasse, a cellulosic waste from cane sugar industry is available in abundant quantities in India and other countries. Its effective hydrolysis into fermentable sugar can meet the scarcity of raw materials for fermentation industries to a large extent. The present work intends to show that bagasse can be saccharified appreciably by proper modifications and enzymic hydrolysis and the hydrolyzate thus obtained can be used for cellular synthesis of food yeasts like *Saccharomyces cerevisiae.*

Materials and Methods

Bagasse: Sugar cane obtained from an agricultural field in Uttar Pradesh was cleaned and shredded by rotary knives followed by extraction of juice by passing through a roller extractor repeatedly. Heavy pressure on the roller coupled with use of water on the partly extracted material made it possible to remove 95–98 per cent of sucrose of the cane. The fibrous residue obtained from this operation is whole bagasse.

Preliminary treatments: The upper hard layer of bagasse was removed and softer cellulosic portion known as pith was cut into small bits which on analysis showed to contain 52 per cent cellulose on dry basis. It was treated with dilute sulphuric acid (2 per cent v/v) at 80°C for two hours with constant stirring to remove pentosans. The fibres were separated by filtration and washed thoroughly with water followed by treatment with 0.1 N NaOH at 80°C for two hours with continuous stirring to remove lignin. After filtration and repeated and thorough washing the fibrous material was dried under sun for eight hours. The dry material was disintegrated first in a waring blender, then in a ball mill and finally screen analysed for different mesh sizes in an electrically driven Rotap testing sieve shaker using 44, 85, 100, 150, 200 and 240 meshes (having particle size range as indicated later) of US Standard Screen for 30 mim.

Enzyme source: The organism used for the production of cellulase was a strain of *Trichoderma viride* QM 6a, obtained from the Department of Food Technology and Biochemical Engineering, Jadavpur University, Calcutta. Culture medium composition was same as described by Mandels *et al*[6]. Two hundred and fifty ml medium at pH 5.3 was sterilized in each of 1 liter Erlenmeyer flasks and was inoculated with 5 per cent (v/v) of inoculum prepared in 10 ml sterile physiological salt solution (0.85 per cent NaCl solution) from a glycerol agar slant culture. Fermentation was conducted at 29°C for 14 days on a reciprocating shaker having amplitude of 3 inch and 96 strokes per min. At the end of fermentation, broth was centrifuged at 900 rpm for 30 min and supernatant containing cellulase was stored at 4°C for use as culture filtrate in saccharification.

Enzyme activity and sugar estimation: Enzyme activity in the culture filtrate was determined by the method of Levinson and Reese[7] and expressed in the same unit of CMC saccharifying activity. The culture filtrate was found to have 35 units of CMC activity per ml. Reducing sugar was estimated by dinitrosalicylic acid (DNS) method[8]. In all experiments sugar was estimated before

Present address: *Biochem. Engg. Division, Indian Institute of Technology, New Delhi-110029
**C.F.T.R.I., Mysore-570013

and after enzymic saccharification of bagasse and the difference is reported in results.

Growth measurements: In the study of cell propagation on bagasse hydrolyzate growth was expressed in terms of optical density (O.D) per ml by measuring with the help of a colorimeter. It is also expressed as the amount of cell mass produced (dry weight) per unit weight of cellulose hydrolyzed. Amount of cellulose hydrolyzed was determined from the difference of the weight of bagasse before and after hydrolysis.

Results and Discussion

Since almost all enzymes exert their optimum activity at a particular pH and temperature, it was necessary to determine the effect of these parameters on enzyme saccharification.

Effect of pH: For observing influence of pH, reaction mixtures containing 5.0 mg of bagasse (240 mesh size) and 5 ml culture filtrate (35 units of activity per ml) were maintained at different pH by adding 5 ml of McIlvain buffer of different pH ranging from 4.5–5.5 and were incubated at 37°C for 4 hr on the reciprocating shaker. After incubation, reaction mixtures were centrifuged to remove unreacted bagasse and supernatant were analysed for reducing sugar per ml of reaction mixture. Results shown in Table 1 indicate optimum saccharification to occur at pH 5.1. Reducing sugar produced at this pH is 0.038 mg per ml reaction mixture corresponding to per cent hydrolysis of 7.6 based on the amount of bagasse used in the reaction mixture.

Effect of temperature: Reaction mixtures containing 5.0 mg bagasse, 5 ml culture filtrate and 5 ml McIlvain buffer at pH 5.1 were incubated for 4 hr at different temperatures as shown in Table 2. Temperatures were maintained on a shaking waterbath. After incubation reducing sugar and per cent hydrolysis at different temperatures were recorded as in Table 2. The optimum temperature requirement for bagasse saccharification by the culture filtrate is 50°C.

TABLE 1. EFFECT OF PH ON THE SACCHARIFICATION OF BAGASSE BY THE CULTURE FILTRATE

Reaction mixture pH	Reducing sugar produced (mg/ml of reaction mixture)	% Hydrolysis
4.5	0.028	5.6
4.8	0.032	6.4
5.0	0.036	7.2
5.1	0.038	7.6
5.2	0.034	6.8
5.3	0.030	6.0
5.4	0.028	5.6
5.5	0.025	5.0

TABLE 2. EFFECT OF TEMPERATURE OF INCUBATION ON SACCHARIFICATION BY CULTURE FILTRATE

Reaction mixture temp. °C	Reducing sugar produced (mg/ml of reaction mixture)	% Hydrolysis
25	0.010	2.0
30	0.012	2.4
37	0.020	4.0
42	0.029	5.8
48	0.035	7.0
50	0.040	8.0
54	0.033	6.6
60	0.027	5.4

A pH of 5.1 and temperature of 50°C corresponds with those of other investigators[9,10].

Effect of substrate to enzyme ratio: Reaction mixtures containing 5 ml culture filtrate, 5 ml McIlvain buffer of pH 5.1 and varying amounts of bagasse (240 mesh size) were incubated at 50°C for 4 hr. After incubation, reducing sugar and per cent of bagasse hydrolysed were estimated. Results are presented in Table 3 from which it can be seen that for optimum saccharification a ratio of 2 mg bagasse per ml of culture filtrate would be most suitable. Higher quantities of bagasse per ml of culture filtrate did not increase the per cent hydrolysis.

Effect of particle size: Typical enzymic reactions involve the dissolution of the substrate to the molecular level whereby maximum efficiency could be expected. Since bagasse is insoluble substrate and because the rate of hydrolysis of cellulose is directly proportional to its amorphous[11] nature the study of the effect of particle size of bagasse on the extent of saccharification is important. Maintaining the same conditions as in previous experiments but keeping the quantity of bagasse constant, studies were carried out with bagasse powder of different particle size. Results are shown in Table 4.

TABLE 3. VARIATION OF SACCHARIFICATION WITH CHANGE IN SUBSTRATE TO CULTURE FILTRATE RATIO

Bagasse in reaction mixture (mg/ml)	Reducing sugar produced (mg/ml reaction mixture)	% Hydrolysis
0.5	0.025	5.0
1.0	0.045	9.0
1.5	0.051	10.2
2.0	0.070	14.0
2.5	0.070	14.0
3.0	0.070	14.0
3.5	0.070	14.0
4.0	0.069	13.8

TABLE 4. PARTICLE SIZE AS A VARIABLE IN SACCHARIFICATION

Bagasse particle size (microns) (×)	Reducing sugar produced (mg/ml reaction mixture)	% Hydrolysis
353<×<178	0.076	7.6
178<×<152	0.092	9.2
152<×<104	0.104	10.4
104<×<76	0.114	11.4
76 <×<66	0.125	12.5
66 <×	0.160	16.0

Reaction mixture consisted of 10 mg bagasse, 5 ml buffer of pH 5.1, and 5 ml culture filtrate (35 units of activity per ml)

It can be seen from Table 4 that as particle size of bagasse decreases the extent of its enzymic saccharification or in other words per cent hydrolysis increases.

Substrate modification: Bagasse obtained by preliminary treatments as described before was subjected further to several kinds of treatments, namely treatment with stronger acid (5 per cent H_2SO_4) and stronger alkali (1 N NaOH) followed by washing, heating (75°C), heating followed by grinding, alkali treatment, neutralization and drying. Acid and alkali treatments were carried out for 2 hr. Heating was carried out for one hour. Reaction mixtures consisting of 10 mg modified bagasse (66<×<76), 5 ml culture filtrate and 5 ml buffer at pH 5.1 were incubated at 50°C for 4 hr after which per cent hydrolysis was determined as described before. Results are given in Table 5.

It seems from the results in Table 5 that for maximum saccharification of bagasse, proper modification through appropriate treatments prior to saccharification is necessary.

Growth of yeast on bagasse hydrolyzate: The liquid containing fermentable sugar obtained by emzymic hydrolysis of bagasse was centrifuged at 900 rpm for 30 min and the clear liquid centrifugate was used to test its suitability as a raw material for cellular synthesis of

TABLE 5. EFFECT OF SUBSTRATE MODIFICATION ON ENZYMIC HYDROLYSIS OF BAGASSE

Type of treatment	Reducing sugar produced (mg/ml reaction mixture)	% Hydrolysis
No treat. after preliminary treat.	0.08	8.0
Acid treated	0.13	13.0
Alkali treated	0.142	14.2
Heat treated	0.122	12.2
Heating and acid treatment	0.150	15.0
Heating, grinding, alkali treat, neutralization and drying	0.240	24.0

TABLE 6. GROWTH OF *S. cerevisiae* ON BAGASSE HYDROLYZATE AND GLUCOSE MEDIUM

Medium	Growth (o.d/ml of medium)	Growth
Enriched bagasse hydrolyzate	7.6	0.32 mg/mg cellulose hydrolyzed
Glucose with other nutrients as in above medium	2.3	0.50 mg/mg glucose utilized

Saccharomyces cerevisiae, a food yeast. In the hydrolytic reaction 2 mg of modified bagasse was used per ml of culture filtrate. The clear hydrolyzate was concentrated by evaporation to give a fermentable sugar concentration of 5.0 mg per ml of centrifugate. This concentrate was autoclaved, cooled and fortified with $MgSO_4$, $7H_2O$, 0.15 mg/ml, $(NH_4)_3$ PO_4, 1.0 mg/ml, Ca–pantothenate, 0.5 mg/ml, thiamine hydrochloride, 4.44 mg/ml and biotin, 0.002 mg/ml under asceptic conditions. It was next inoculated with 5 per cent inoculum of the organism prepared in the same way as described in the case of *Trichoderma viride* QM6*a* and incubated at 28°C on a reciprocating shaker, having 3 inch amplitude and 96 strokes per min, for 18 hr. After incubation growth of the organism was measured. For comparison analytical grade glucose was used in place of concentrated bagasse hydrolyzate in growth medium. The results shown in Table 6 indicate that yeast growth in pure glucose medium is higher, compared with that in bagasse hydrolyzate medium. This lower growth in hydrolyzate might be due to the presence of certain hydrolytic product which is inhibitory to the growth of yeast.

Conclusion: Informations on enzymic saccharification of bagasse provided in this paper would be useful in commercialization of the process. Results of the effect of process variables like pH and temperature for exertion of optimum activity of cellulase systems of *T. viride* QM6*a* in bagasse saccharification was in conformity with the findings of other workers[9,10]. Enhancement of saccharification by size reduction of bagasse particles and its modifications by chemical and physical treatments establish the necessity of proper pretreatment for optimum saccharification of bagasse. This is in agreement with the report of some worker[5] with different cellulosic material. The extent of saccharification by acid hydrolysis (7 per cent sulphuric acid solution) has been reported to be 2.9 per cent[12]. In contrast 24 per cent hydrolysis has been obtained under optimum conditions worked out in these experiments. Usefulness of bagasse hydrolyzate as substrate for single cell protein production has been reported[3]. We have also found that it can be used in the production of *S. cerevisiae*, a

food yeast. It indicates the possibility of substituting hydrocarbons by this hydrolyzate as substrate in SCP production. Since hydrocarbons have been reported to impart carcinogenic property to the SCP obtained by the use of hydrocarbons bagasse can be hydrolysed and utilized as suitable substrate.

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ERRATA

"Studies on pectin methyl esterase activity during development and ripening of guava fruit" by Pratima N. Sastri and N. V. Sastri, published in this Journal 1975, **12** (1), 42.

(*A*) Page 42, column 1, para 2, line 9.
Matter printed as COOH groups liberated/hr/g. should be read as COOH groups liberated/hr/10 g.

(*B*) Page 42, Figure 1.
Curve 'c' represents total pectin content (mg ca pectate/100 g fresh weight). The second vertical line on the right hand side of the figure represents this parameter (range 0–800 mg).

Controlled Sun Drying of Freshly Harvested Paddy for Improved Milling Quality

M. K. Bhashyam, T. Srinivas and H. S. R. Desikachar
Central Food Technological Research Institute, Mysore India.

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Freshly harvested paddy at 20-24% moisture in several varieties was sun dried under different weather conditions. The effect of stirring and intermittent covering (tempering) during drying on the milling quality of paddy was evaluated. It was observed that when the temperature was high (40–45°C) and humidity was low (less than 45%) direct sun drying caused high breakage. Stirring the paddy once in about half an hour and inclusion of one or two tempering steps when grain reaches 17% moisture was highly beneficial. When the temperature is mild (25–32°C) the tempering steps may not be essential. A general recommended procedure is to dry the paddy upto 17% moisture with stirring at half hour intervals followed by a two to three hour tempering under cover and final drying to about 14% moisture.

The detrimental effect of late harvesting of paddy on its milling quality is well known[1–6]. For minimising milling breakage, therefore, harvesting of paddy at an average moisture content in the range of 20–24 per eent has been suggested and has been increasingly practised in many rice producing countries. Mechanical driers have been used, as a rule, for drying of the paddy in the developed countries and for handling bulk quantities of paddy in the large assembly centres even in the developing countries. The land holding situation in many Indian villages does not however favour the widespread use of such mechanical driers and calls for a simple, relatively inexpensive method of drying paddy without affecting its milling quality. In regions where fair weather is present at the time of harvesting, sun drying promises such a possibility provided the deficiencies of sun drying as customarily practised can be overcome.

Craufurd[7], working in Africa, recommended that paddy could be dried with minimum damage to milling quality by reducing the severity of the African sun by interposing a light shading between the sun and the paddy and not allowing paddy temperature to rise beyond 38°C. Chancellor[8] arrived at equations from which drying rate of paddy could be predicted and his work showed that drying rate depended on area of paddy exposed to solar radiation. Frequent raking improved the rate of drying. Bhattacharya and Ali[9] improved on the traditional method of sun drying of parboiled paddy and stressed the usefulness of an intermediate tempering step found so useful in mechanical drying of paddy for minimising milling breakage. The application of the principles of (*i*) frequent raking for improving the uniformity of drying, and (*ii*) tempering for moderating drying rate for better milling quality for drying of freshly harvested paddy and standardising a workable procedure for adoption by farmers in Indian villages is reported here.

Materials and Methods

Paddy was harvested when average grain moisture was 20–24 per cent threshed immediately and freed of chaff. The paddy was spread in 1 inch thick layer on a paved yard and dried for about 2 hr when the moisture level was reduced to 15–18 per cent. The paddy was raked and mixed every half an hour during this period. This paddy was divided into 3 lots and processed as follows: One lot was dried continuously without stirring, the second lot dried with stirring once in every half an hour and the third sample was covered with a tarpaulin for one hour followed by drying for half an hour alternately until grain moisture was reduced to about 14 per cent. Samples were withdrawn at intervals and moisture content determined by oven drying method by drying at 105°C for 16 hr. All samples were shade dried to about 14 per cent moisture for milling. Milling quality was determined in a McGill type laboratory sheller[10].

Based on this initial explorative study conducted at Hiriyur (with air temperature, 39 to 44°C and RH 44 to 20 per cent) on one variety (Halubbulu), experiments were carried out in May 1972 on 4 other varieties at Gangavathi Farm, Karnataka State where environmental temperature varied from 29 to 37°C, ambient RH varying from 60 to 40 per cent. In January 1973 these studies were extended to 3 other varieties at Nagenahalli Farm, Mysore where ambient air temperatures were lower (24 to 30°C, the RH varying from 60 to 40 per cent). In order to study the effect of drying with higher air tem-

peratures two more varieties were sun dried during the summer of 1973 at the Bhavanisagar Farm, Tamil Nadu State(34 to 39°C and RH 65 to 61per cent).

Results and Discussion

Data from studies on the drying characteristics of paddy are presented in Fig. 1-4. Typical data on one variety in each of the four sets of experiments are presented. As can be seen from the curves presented in Figl-4, continuous drying of the paddy allowed drying in a short period but caused higher breakage than when the drying was slowed down by tempering or holding in between drying steps. The beneficial effect of stirring once in half an hour is evident from the data in Fig. 1. Summary of milling breakage data for each of the different varieties is presented in Table 1.

When the environmental temperature was mild (25–32°C) as in the winter experiments at Nagenahalli the differences between the shade dried and the sun dried paddy samples tended to become very small indicating that continuous drying may be possible in such environments. However when the ambient temperature was high (35–40°C) as in Hiriyur or Bhavanisagar or when the paddy had poor milling quality due to varietal peculiarity as in Jaya and MCM–2 the difference between the control shade dried paddy and the sun dried paddy samples was large. In such cases the inclusion of one or more tempering step would be beneficial.

Although three or more tempering steps have been included in many of the experiments represented in Fig. 1-4, further studies have shown that two tempering steps are quite sufficient as milling quality of paddy was essentially similar between the two step or four step tempering procedure. For varieties with fairly good milling quality (S—701, IR-20 and S—749) even one tempering step of 3-4 hr is practicable without causing

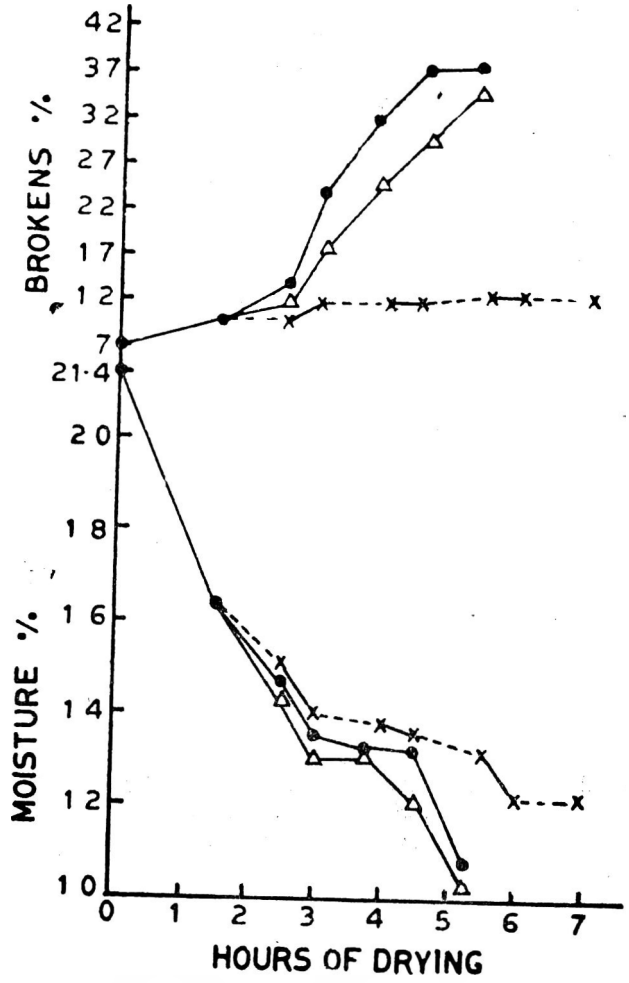

Fig. 1. Effect of different methods of sun drying on milling breakage of Halubbulu paddy at Hiriyur.

—△— Sun drying with stirring; —●— Sun drying without stirring; —×— Drying phase; --×-- Tempering phase under cover.

TABLE 1. EFFECT OF SUN DRYING ON MILLING BREAKAGE OF PADDY

Variety & place	Season	Ambient air temperature range (°C)	Ambient* RH range, %	Initial paddy moisture %	Milling breakage %		
					Shade dried	Continuous drying	Dried with tempering
S–701 (N)	Jan 73	25–30	57–47–44	21.0	6.0	9.5	6.2
S–1092 (N)	Jan 73	29–32	55–60–57	20.0	22.5	25.2	25.0
S–749 (N)	Jan 73	24–30	58–42	20.0	15.2	18.6	16.8
Jaya (N)	Jan 71	25–32	34–40	18.4	26.0	45.0	31.0
MR–27 (G)	May 72	29–37	60–42–39	22.0	39.0	44.0	43.0
MCM–2 (G)	May 72	29–37	60–42–39	25.5	38.0	57.0	53.0
CO–29 (G)	May 72	29–37	60–42–39	22.0	14.2	19.0	18.0
IR–20 (G)	May 72	30–42	67–47–32	19.0	20.0	28.0	22.0
IR–20 (B)	May 73	30–42	65–61–61	24.0	16.0	22.0	16.9
Kanchi (B)	May 73	34–39	65–61–61	21.9	26.5	28.5	28.0
Halubbulu (H)	May 71	39–44	44–22–20	21.4	7.0	38.0	13.0

N–Nagenahalli, Karnataka; G–Gangavati, Karnataka; B–Bhavanisagar, Tamil Nadu; and H–Hiriyur, Karnataka.
*Relative humidity (RH) was measured at 10 a.m., 12 noon and 4 p.m.

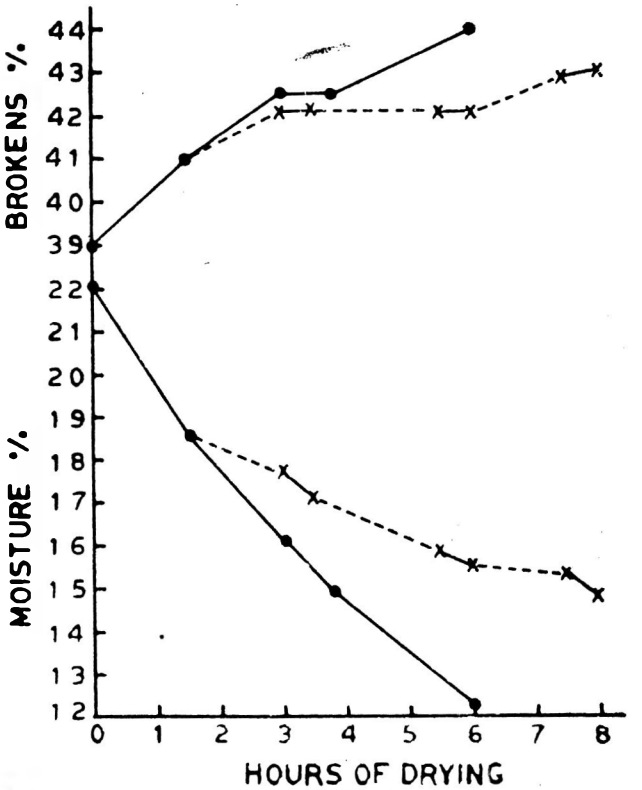

Fig. 2. Effect of different methods of sun drying on milling breakage of MR-27 paddy at Gangavati.
Legend as under Fig. 1.

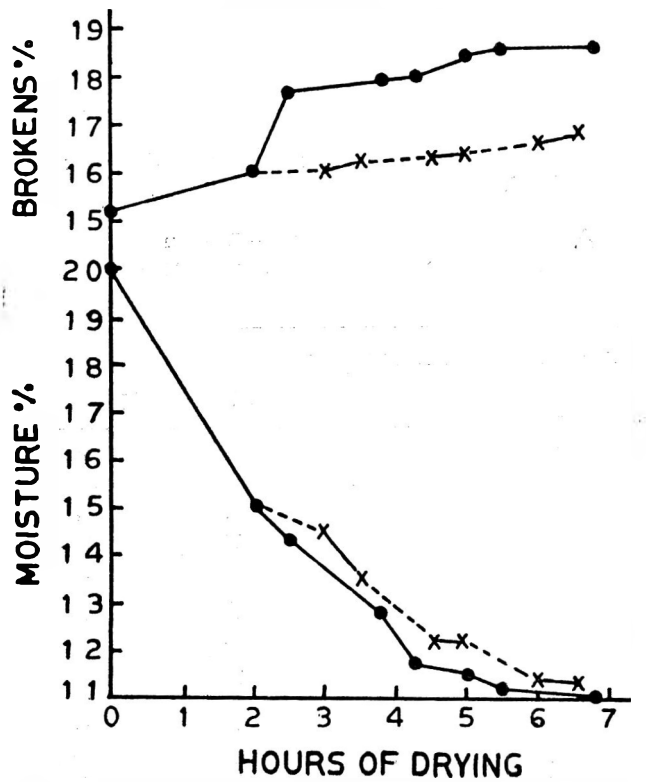

Fig. 3. Effect of different methods of sun drying on milling breakage of S-749 paddy at Nagenahalli.
Legend as under Fig. 1.

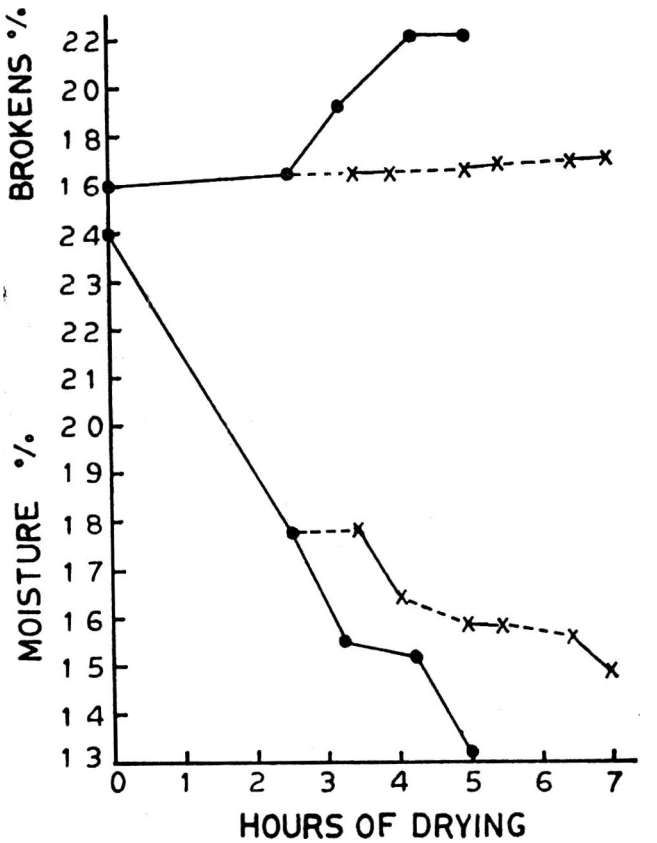

Fig. 4. Effect of different methods of sun drying on milling breakage of IR-20 paddy at Bhavanisagar.
Legend as under Fig. 1.

undue increase in breakage of paddy. The type of covering to be provided to the paddy is not highly critical and could be locally provided. It could be a tarpaulin, mat, straw or coconut leaf cover to prevent exposure of the paddy to the direct sun. In studies to find out the essentiality of heaping during the tempering period, it has been shown that the paddy could even be spread during the tempering phase but has to be under cover against sun. Moisture determinations show that slow drying continues to occur even under such covered conditions.

The major winter harvesting season for paddy is dry and clear over a larger part of India and sun drying would be practicable. The summer paddy season is also normally hot and dry during the day although for the late harvested summer crop and the 'Kuruvai' crop of Tamil Nadu the weather is monsoon type and unfavourable. The present studies show that wherever clear weather prevails, sun drying can be practised with minimum damage to the milling quality of paddy provided the scientific principles enumerated above are followed and appears to be the most economical method of drying practicable for small farmers in the villages.

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Effect of Washing, Cooking and Dehydration on the Removal of Some Insecticides from Okra (*Abelmoschus esculentus* Moench.)

G. NATH*, N. R. JAT AND B. P. SRIVASTAVA
Department of Entomology, University of Udaipur, Rajasthan, India.

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Malathion residue on *bhindi* (okra) was removed by 89 and 79 per cent due to washing, 87 and 58 per cent due to open-cooking, 76 and 58 per cent due to steam-cooking, 92 and 92 per cent due to dehydration from lower (0.05%) and higher (0.1%) treatment dosages respectively. Thus all the processes removed malathion residue below the tolerance limit of 3 ppm.

In case of carbaryl treated vegetables washing removed the residue by 66 and 70 per cent, open cooking by 42 and 25 per cent, steam- cooking by 82 and 49 per cent, dehydration by 79 and 77 per cent from lower (0.25%) and higher (0.5%) application dosages respectively indicating thereby that in case of lower treatment dosage only all the processes except open-cooking removed the residue below the tolerance limit of 10 ppm.

Endosulfan residue due to the application dosages of 0.1 and 0.2 per cent was removed by 35 and 49 per cent by washing, 26 and 24 per cent by open-cooking, 26.93 and 31.12 per cent by steam-cooking, 58 and 57 per cent by dehydration respectively. Thus, none of the processes in any of the two treatment dosages could remove the residue below the prescribed tolerance limit of 2 ppm.

Some important insect pests of Okra (*Abelmoschus esculentus* Moench.) particularly the shoot and fruit borers (*Earias fabia* Stoll.) jassids (*Emrasca devastans* Dist.) and aphids (*Aphis gossypii* Glover) are major limiting factors in the profitable cultivation of this crop. Naturally, therefore, insecticidal sprays are frequently used on this crop for its protection from the ravages of the insect pests. 'Pusa sawani' variety of okra which is often recommended to cultivators due to its high yielding character, starts bearing when the plants are about 6 inches tall and continues fruiting. The vegetables that receive insecticidal deposits due to foliar sprays reach the market for human consumption. Malathion, carbaryl and endosulfan have been recommended to control the pests of okra crop by Lal and Dhall[1], Satpathy and Mishra[2], Jat and Srivastava[3], and Dadheech[4]. The residues of these insecticides on okra may cause health hazards to the consumers particularly if the residues are more than the prescribed tolerance. Since okra are never eaten raw and are

Present address: Department of Zoology, University of Allahabad.

often washed, cooked or dehydrated before use, the objective of the experiment was to examine the effect of these processes on the removal of the residues of malathion, carbaryl and endosulfan which may remain on this vegetable in more than desirable quantities if proper time interval between application and harvest is not adopted. The initial deposits received from the above insecticides were taken as the base for examining the effect of various processes. The results of the studies conducted from this point of view are reported in the present paper.

Materials and Methods

Technical grades of malathion, carbaryl and endosulfan were used for preparing standard calibration curves (after mathematical extrapolation) using distilled carbontetrachloride, acetone and isopropyl alcohol (w/v) respectively as the solvents. The commercial formulations of different insecticides were used as foliar sprays for field application. Spray solutions containing 0.05 and 0.10 per cent malathion, 0.25 and 0.50 per cent carbaryl and 0.10 and 0.20 per cent endosulfan (v/v) were prepared in ordinary tap water.

The crop was raised on the Horticulatural Farm of Rajasthan College of Agriculture, Udaipur. The experiment was laid out in a randomized block design. The size of each plot was 8 × 4 meters. The seeds of okra variety 'Pusa sawani' were sown at the rate of 18 kg-ha. The crop was sprayed 60 days after sowing when sufficient number of fruits had appeared. A hand compression sprayer was used for spraying which was calibrated before use. Spraying was done to the dripping level.

The samples of normal marketable okra (3-4") were randomly drawn from each treated and control plot, 1-2 hr after the spraying. The samples were collected with the help of scissors in previously tagged polythene bags without touching by hand. In order to find out the initial deposits (residues before processing) the samples were chopped in 0.5 to 1 cm sizes with scissors. Representative samples of 50 g from each treatment and control were put into properly labelled wide mouth 500 ml conical flasks. Extraction of malathion was done with 100 ml carbontetrachloride and that of carbaryl with 150 ml of dichloromethane by vigorous shaking for an hour on an electric shaker. The extract was filtered over anhydrous sodium sulphate placed in a funnel plugged with cotton wool into labelled bottles which were made air tight and stored in deep freezer for further analysis. In case of endosulfan treated fruits the extraction of chopped fruits was done with methyl cyanide (acetonitrile) by blending for 1 min and filtered through cintered funnel by vacuum pump. The volume of each 50 g sample extract was made to 150 ml. The contents were rinsed with 200 ml of normal hexane and filtered again. The filtrate was added to the methyl cyanide extract.

In order to examine the effect of washing, normal marketable fruits were washed for 30 sec with tap water before chopping for extraction. To study the effect of different methods of cooking on the removal of residues the vegetable was chopped and cooked for 10 min both by open cooking and steam (pressure) cooking respectively before they were extracted with their respective solvents in the manner described above. To study the effect of dehydration the normal marketable samples were cut lengthwise into 4 quarters and blanched with boiling water for 4 min. They were then dried at 68°C for 8 hr. After desired degree of dehydration (drying ratio of 2:1) the fruits were extracted with their respective solvents in the manner described earlier.

The estimations of microquantities of residues were carried out by the colorimetric method. The method used for malathion was the one employed by Norris *et al.*[5] and modified and recommended by Malathion Panel[6]. Carbaryl analysis was done by the method given by Benson and Finochiaro[7] which is a modification of official colorimetric method[8]. Endosulfan analysis was done by the method recommended by Butler *et al.*[9] and modified by Maitlen *et al.*[10]. Recovery in the fortified okra with malathion, carbaryl and endosulfan was found to be 71.66, 80.81 and 78.88 per cent respectively. The per cent reduction of residues was calculated on the basis of the initial deposit (residue before processing) and the residue obtained after each individual processing.

Results and Discussion

The normal marketable size of okra fruits received initial deposits of 2.58 and 4.63 ppm for malathion 0.05 and 0.10 per cent sprays, 17.53 and 40.20 ppm for carbaryl 0.25 and 0.5 per cent sprays, 6.98 and 15.10 ppm for endosulfan 0.1 and 0.2 per cent sprays. These initial deposits were taken as base to examine the effect of various processes on the extent of their removal.

Effect of washing: Only a 30 sec washing of treated okra with tap water resulted in considerable removal of malathion deposits which is evident from the fact that a residue of 0.28 and 0.95 ppm of malathion was found on washed okra for an application dosage of 0.05 and 0.10 per cent malathion, recording thereby a reduction of 89.15 and 79.48 per cent respectively. Wallis *et al*[11]. also reported that only one minute washing of okra having an initial deposit 15.20 ppm of malathion removed the residue to the extent that only traces of malathion could be detected on washed okra In case of tomato, Singh and Lal[12] reported a reduction of 86.20 per cent in malathion deposit by one minute

TABLE 1. EFFECT OF WASHING, COOKING AND DEHYDRATION ON THE REMOVAL OF THE DEPOSITS OF MALATHION, CARBARYL AND ENDOSULFAN FROM OKRA

Treatment dosage %	Initial deposit	Residue after washing		Residue after open cooking		Residue after steam cooking		Residue after dehydration	
	Replicates (ppm)	Replicates (ppm)	Reduction (%)	Replicates (ppm)	Reduction (%)	Replicates (ppm)	Reduction (%)	Replicates (ppm)	Reduction (%)
				MALATHION					
	2.92	0.30		0.42		1.20		0.20	
0.05	2.96	0.35	89.15	0.35	86.82	0.41	75.97	0.25	91.86
	1.85	0.19		0.25		0.25		0.19	
	(2.58)	(0.28)		(0.28)		(0.62)		(0.21)	
	3.80	0.30		1.95		1.80		0.40	
0.1	5.39	1.70	79.48	2.40	58.32	2.20	57.88	0.40	91.79
	4.70	0.85		1.43		1.85		0.35	
	(4.63)	(0.95)		(1.93)		(1.95)		(0.38)	
				CARBARYL					
	18.20	5.94		10.20		4.20		4.44	
0.25	16.20	5.64	66.12	10.20	41.81	2.70	81.75	2.64	79.24
	18.20	6.24		10.20		2.70		3.84	
	(17.53)	(5.94)		(10.20)		(3.20)		(3.64)	
	40.20	12.24		29.70		22.20		11.64	
0.5	34.20	11.64	69.55	29.70	24.88	20.70	48.51	6.84	77.01
	46.20	12.84		31.20		19.20		9.24	
	(40.20)	(12.24)		(30.20)		(20.70)		(9.24)	
				ENDOSULFAN					
	6.45	4.44		4.84		4.80		2.80	
0.1	6.98	4.44	34.67	5.24	26.22	5.26	26.93	2.84	57.59
	7.52	4.80		5.36		5.24		3.24	
	(6.98)	(4.56)		(5.15)		(5.10)		(2.96)	
	14.98	7.64		11.24		10.04		6.20	
0.2	13.12	8.64	48.94	11.84	23.58	11.61	31.12	6.64	57.35
	17.20	6.84		11.54		9.85		6.48	
	(15.10)	(7.71)		(11.54)		(10.50)		(6.44)	

Figures in parenthesis indicate mean values.

washing only. Elkines *et al.*[13] also reported that cold water washing removed 96 per cent malathion residue from beans.

In case of carbaryl the initial deposit as a result of two application dosages reduced to 5.94 and 12.24 ppm respectively by tap water washing of okra for 30 sec (Table 1). Thus, washing decreased the carbaryl deposit by 66.12 and 69.55 per cent for lower and higher application dosages respectively. Deshmukh and Lal[14] also reported that tap water washing of brinjal treated with carbaryl removed its residue to a great extent and the residue amounting to 48.50 ppm was reduced by washing below prescribed tolerance limit. Dewan *et al.*[15] working on okra also reported that 11.83 cm rainfall washed the initial deposit of carbaryl to nil thereby recording a 100 per cent reduction of carbaryl residues. Bindra[16] reported 80 to 83 per cent reduction of carbaryl by washing of tomato.

It is interesting to note that in case of endosulfan 30 sec tap water washing resulted only in 34.67 and 48.94 per cent removal of the endosulfan deposits as the amount of residue detected was 4.56 and 7.71 ppm for lower and higher treatment dosages respectively, indicating thereby that endosulfan is more persistant and is not easily removed by washing. Bindra[16] also reported that in case of tomato, only 18 to 55 per cent endosulfan could be reduced by washing.

It is thus evident from the above that only half a

minute washing of vegetables treated with either of the doses of malathion removes their deposit for below the FAO/WHO[17] prescribed tolerance of 3 ppm. However, in case of carbaryl washing removed the residue below the FAO/WHO tolerance limit of 10 ppm in case of lower treatment dosage only. Endosulfan proved to be far more persistant to washing and in both the treatment dosages the residue after the same amount of washing was far above the FAO/WHO prescribed tolerance of 2 ppm. Bindra[16] also found endosulfan to be more persistent to washing than carbaryl on tomato and malathion on cabbage.

Effect of cooking: The data indicate that after 10 min open and steam cooking of okra treated by 0.05 per cent malathion, only a residue of 0.34 and 0.62 ppm malathion was found on okra recording thereby a decrease of 86.82 and 75.97 per cent respectively. However, in case of 0.10 per cent malathion spray the initial deposit was reduced to 1.93 and 1.95 ppm by open and steam cooking recording thereby a reduction of 58.32 and 57.88 per cent respectively. The percentage reduction by both the processes was less in case of higher treatment dosage both by open cooking and steam cooking. Elkines *et al.*[13] reported that in case of home cooked beans only traces of malathion could be detected.

The same amount of open and steam cooking of okra treated with 0.25 per cent carbaryl reduced the residue to 10.20 and 3.20 ppm recording thereby a decrease of 41.81 and 81.75 per cent respectively. However, in case of 0.50 per cent application dosage the initial deposit was reduced to 30.2 and 20.7 ppm by open and steam cooking respectively. Thus, the reduction was found to be 24.88 and 48.51 per cent by open and steam cooking. It is evident from the above that in both lower and higher application dosages the reduction percentage was twice as much by steam cooking as compared to open cooking. Farrow *et al.*[18] working on carbaryl residues in tomato reported that home cooking removed 96 per cent of carbaryl residues while Elkines *et al.*[13] could detect only the traces of carbaryl from beans after home cooking.

In case of endosulfan open and steam cooking of Okra treated with 0.1 per cent reduced the deposits to 5.15 and 5.10 ppm recording thereby a decrease of 26.22 and 26.93 per cent respectively. However, in case of 0.2 per cent application dosage the initial deposit was reduced to 11.54 and 10.50 ppm by open and steam cooking respectively. The reduction was found to be 23.58 and 31.12 per cent for lower and higher application dosage. Thus, in case of both application dosages the extent of removal by open and steam cooking was, more or less, similar.

It is evident from the above that both open and steam cooking removed the deposit from both treatment dosages of malathion below the tolerance limit of 3 ppm and both processes removed almost the same amount. In case of carbaryl, steam cooking removed about double the deposit as compared to open cooking for both the treatment dosages. However, in case of endosulfan the amount of removal was almost equal by both the processes. None of the two processes, except steam cooking for lower treatment dosage of carbaryl, could bring the carbaryl and endosulfan residue below the tolerance limit of 10 ppm and 2 ppm respectively.

Effect of dehydration: Dehydration of treated okra resulted in the removal of malathion deposit by 91.86 and 91.79 per cent as a residue of 0.21 and 0.38 ppm could be detected on dehydrated for lower and higher application dosages respectively.

Dehydration resulted in the removal of carbaryl deposit by 79. 24 and 77.01 per cent as a residue of 3.64 and 9.24 could be detected on dehydrated fruits for lower and higher application dosages respectively.

In case of endosulfan the dehydration resulted in 57.59 and 57.35 per cent removal of the initial deposit as the amount detected was 2.96 and 6.44 ppm for lower and higher treatment dosages respectively.

It is evident from the above that the extent of removal of the deposits by dehydration from both treatment dosages for all the three insecticides was more or less, similar. Moreover, dehydration brought down the residue below prescribed tolerance limits in case of both treatment dosages of malathion and carbaryl, while the endosulfan residue after dehydration was more than the tolerance of 2 ppm in case of both treatment dosages.

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Relative Amylase Activity of Some Malted Cereal Grains

A. K. JAIN AND W. B. DATE
Biochemical Engineering and Food Technology Research Laboratory, H. B. Technological Institute, Kanpur, India

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Malting properties of jowar and Bajra were studied. Diastatic power developed during germination is rapid as compared to that of Barley. However, this property subsequently declined after 32 hr whereas in barley it continued to increase upto 72 hr.

Good amylase activity of a grain is desirable to obtain solubilisation of starch and its subsequent conversion to maltose. All cereal grains do possess this property, however, the degree varies. Sheorain and Wagle[1] have reported β-amylase activity of Bajra recently. In order to assess the relative amylase activity and to indicate the possible use of coarse grains for malt and maltose preparation the present investigation was undertaken. Barley is conventionally considered as suitable raw material for malt in brewing industry. However, availability of Barley is not adequate. To fill up the gap in the total demand of good quality malt, raw materials other than barley deserve investigation.

Materials and Methods

Two varieties each of Jowar (*Sorghum vulgare*), (Type-4 and Type-8B) and of Barley (Clipper-two row and Vijya-six row) were obtained from the Cereal Botanist at the U. P. Institute of Agricultural Sciences, Kanpur. Bajra (*Pennisetum typhoideum*) of unspecified variety was procured from local market because selection of this was not available. All the seeds were analysed for moisture, protein, starch and ash using AOAC method[2]. To check the effect, if any, of pretreatment on germination and subsequent enzyme activity dilute solutions of calcium nitrate, gibberellic acid[2] and only water as control were used for soaking seeds. These chemicals are known to promote growth. The temperature during steeping was manually controlled within a range of 15–21°C.

After soaking, seeds were placed on wet filter paper in petridishes for germination. Kilning was done at different temperatures and at different stages of germination. Initially the temperature was kept at 35°C and it was raised to 85°C in 10 to 12 hr. After grinding and screening to remove husk, the samples were stored in air tight containers at room temperature for subsequent assessment of the relative diastatic activity. To express diastatic values Degree Linter, a unit of amylase activity was used[3–5]. A Linter unit is, "the number of grams reducing substances calculated as maltose produced by 25 g of malt in half an hour digestion of soluble starch at 20°C".

TABLE 1. IMPORTANT CONSTITUENTS OF GRAINS USED FOR MALTING

Grains	Variety	Moisture %	Ash %	Protein %	Starch %	Wt. of 1000 seeds (g)
Barley	Clipper	13.2	1.83	10.8	59	46.76
	Vigya	10.7	2.19	10.4	63	43.29
	Type-4	6.8	1.46	11.5	57	21.20
Bajra	Unspecified	9.8	1.34	12.5	60	18.93

TABLE 2. EFFECT OF PRETREATMENT ON GERMINATION AND WATER CONTENT OF GRAINS

Grain	Variety	Pretreatments: Dist. water		0.5% Urea		0.5% Cal. nitrate		0.5% Amm. nitrate		0.5 ppm GA	
		Ger. %	I. W. %	Ger. %	I. W. %	Ger. %	I. W. %	Ger. %	I. W. %	Ger. %	I. W. %
Barley	Clipper	35	39.8	52	38.7	55	39.9	41	38.4	63	39.5
	Vigya	92	45.4	89	43.3	95	42.6	89	42.4	98	44.3
Jowar	Type-8B	82	43.3	17	45.8	57	44.9	56	45.0	80	45.1
	Type-4	80	36.5	36	41.1	83	44.0	85	39.1	83	40.2
Bajra	Unspecified	90	44.2	87	43.4	80	40.1	92	45.0	99	43.9

For all the above observations a temperature of 21°C and duration 26 hr was provided.
Ger = Germination; I.W. = % Increase in water; GA = Gibberellic acid.

TABLE 3. DIASTATIC POWER GENERATED DURING GERMINATION (IN LINTER UNIT °L)

Grain	Variety	Sampling time (hr)	Diastatic power in: Dist. water	0.5% Cal. nitrate	0.5 ppm GA
Barley	Clipper	8	23	25	30
		32	62	62	67
		72	98	99	103
	Vigya	8	35	39	52
		32	102	102	113
		72	132	135	140
Jowar	Type-8B	8	131	133	135
		32	168	170	176
		72	113	115	130
	Type-4	8	130	131	135
		32	169	170	179
		72	113	115	120
Bajra	Unspecified	8	130	133	136
		32	170	170	176
		72	116	117	120

GA = Gibberellic acid.

Results and Discussion

The chemical analysis for some important constituents of barley, jowar and bajra are given in Table 1. Data on effect of pretreatments with chemicals on germination are given in Table 2. The diastatic power developed during germination is presented in Table 3. It is seen that moisture, ash and starch contents of jowar and Bajra were lower than those of barley. While studying the nature of diastatic activity in barley, Jowar and Bajra, when water only was used as steeping liquid, it was observed that diastatic activity in Bajra and jowar reached a maximum value after 32 hr of germination and it was more than that of barley. However, it rapidly declined and at 72 hr it was practically nil. The enzyme activity in barley, however, showed a comparatively slow development upto 8 hr germination. This activity rose rapidly between 8 to 32 hr and continued to increase gradually to 72 hr of germination. As regards effect of chemicals, added to soaking baths, it was seen that gibberellic acid and calcium nitrate appeared to be helpful in the promotion of diastatic acitivity. Gibberellic acid was better than calcium nitrate (Table 3.)

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Incorporation of Sunflower Seed Meal in Bread

N. C. Jain, N. D. Shiralkar and B. Y. Rao
Laxminarayan Institute of Technology, Nagpur University, Nagpur

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Sunflower seed meal is a good protein source which can be incorporated in bread. Breads were prepared by replacing wheat flour to the extent of 10, 15 and 20% with defatted sunflower seed meal. The resulting breads showed good crust, crumb, grain size, texture and improved taste at 10 to 15% replacement. A slight change in colour of the crumb was observed which may be due to phenolic compounds present in the meal.

Bread is consumed universally as a food. Attempts are being made to fortify bread with various ingredients like proteins, vitamins, minerals, etc. The partial replacement of wheat flour with oilseed meal which are high in protein contents is one such method to augment the protein supply and to improve the nutritional quality of the product.

It is well recorded in the literature that non-glutenous protein adjuncts exert a volume depression effect along with serious deterioration of crumb, colour, grain and texture, when used at the relatively high level necessary to accomplish the desired amount of fortification[1,2]. The maximum level of replacement depends on the type of non-wheat flours, the baking procedure and the dough stabilizing compounds used[3,4].

Sunflower proteins are of high quality and no anti-biological constituents have so far been reported[5]. Some workers[6] have incorporated defatted sunflower seed meal substituting 3-20 per cent wheat flour with no adverse effect on flavour and colour of the loaf, but the loaf volume was severely affected. In the present work the defatted sunflower seed meal has been incorporated in the formulation of bread and the results of partial substitution of wheat flour on the loaf volume and texture have been reported.

Materials and Methods

Preparation of defatted sunflower meal (DSM): Sunflower meal was prepared by solvent extraction of oil from kernels. The air dried meal is toasted at about 60–80°C in an air oven. The meal was ground and screened through 60 mesh sieve. The proximate analysis of the sunflower meal (DSM), wheat flour (*Maida*) was carried out according to AOAC methods[7]. Chlorogenic and caffeic acids were estimated by quantitative paper chromatography[8]. The results are given in Table 1.

Preparation of bread: The breads were prepared by straight-dough method[9]. Recipe for standard and fortified breads are given in Table 2.

Preparation of the dough and baking: One kg each of 10, 15 and 20 per cent level blends of defatted sunflower meal with wheat flour were taken for the preparation of bread. The dough was prepared by mixing all the ingredients thoroughly and kneaded by hand. Dough mixing time was about 10 min. Dough was fermented for about an hour at room temperature(30°C). Dough was then scaled to one pound and rested for 30 min in moulds. All the samples prepared were then baked simultaneously for a period of half an hour in a country *Bhutti* which is used by local bakers. Baking temperature was about

Table 1. Composition of the principal ingredients used in the study

Composition %	*Maida*	Defatted sunflower meal
Moisture	12.2	4.60
Protein	10.4	49.50
Ash	2.1	4.50
Chlorogenic acid	—	1.65
Caffeic acid	—	0.30

Table 2. Recipe of bread with different blends

Ingredients	Control	Sunflower bread		
g.	0	1	2	3
Maida	100	90	85	80
Defatted sunflower meal	0	10	15	20
Vanaspati	3	3	3	3
Sugar	6	6	6	6
Salt	1.5	1.5	1.5	1.5
Yeast	2.8	2.8	2.8	2.8
Water (ml)	70	60	60	60

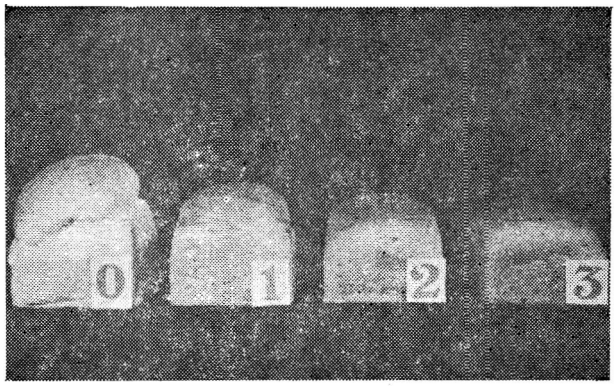

Fig. 1. Bread prepared by incorporating sunflower meal. 0: Control; 1, 2 and 3 indicate 10, 15 and 20 per cent sunflower meal respectively.

221°C. The bread loaves were cooled for 20 min, weighed and the loaf volume was determined by rapeseed displacement[10].

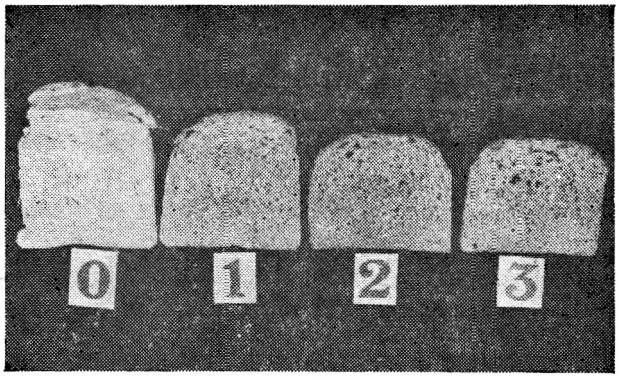

Fig. 2. Cross section of the bread. Legend same as in Fig. 1.

Results and Discussion

The texture of the bread depends upon the loaf volume and spongeness or elasticity and the quality and quantity of gluten and moisture content. When the defatted sunflower meal is incorporated in the bread the gluten content of the mixture is decreased. Experience has shown that if more water is added to the flour, the dough becomes sticky. Therefore water content was adjusted to 60 per cent on the weight of the flour. The loaves prepared by addition of sunflower meal, designated as 0, 1, 2, 3 for control, 10, 15 and 20 per cent addition of DSM respectively are shown in Figs. 1 and 2.

TABLE 3. PROXIMATE ANALYSIS OF THE LOAF

Composition %	Control	10% DSM	15% DSM	20% DSM
Moisture	40.0	37.1	36.5	35.1
Protein	6.0	10.0	10.9	12.5
Oil	2.9	2.6	2.3	1.8
Ash	3.0	3.5	3.8	4.2
Carbohydrate*	48.1	46.8	46.5	46.5
Loaf volume (ml)	1540	1320	1290	1000
Specific volume (ml/g)	3.27	3.07	3.07	2.38

*By difference including crude fibre.

The results of these trials (Table 3) show that increased percentage of defatted sunflower meal (DSM) decreased the loaf volume. The specific volumes for incorporation of 10 and 15 per cent DSM remained unchnaged indicating that there is no significant change in the loaf volume by increasing the level of fortification from 10 to 20 per cent. At the same time protein content is increased from 10 to 12.5 per cent without affecting the loaf quality. The crust of the bread was attractive (Fig. 1). The crumbs of these breads were also good with uniform grain size and texture. However the colour of the crumb was grey. This may be mainly due to polyphenolic compounds in sunflower meal. These compounds are recognised as chlorogenic, caffeic and quinic acids. The chlorogenic and caffeic acid contents of the sunflower meal were estimated to be 1.65 and 0.3 per cent respectively. These phenolic compounds when heated form oxidised products and cause discolouration. But the taste of the bread was better than that of control.

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Studies on Tempeh: Part I. Processing and Nutritional Evaluation of Tempeh from a Mixture of Soybean and Groundnut

R. Girija Bai, T. N. Prabha, T. N. Ramachandra Rao, V. P. Sreedhara and N. Sreedhara
Central Food Technological Research Institute, Mysore, India

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A process for the preparation of Tempeh (a fermented soybean food) with a mixture of groundnut and soybean was standardised. Tempeh was prepared with three strains of *R. oligosporus* NRRL 2710, 2549 and 514. The two types of Tempeh prepared were analysed for ash, moisture, crude fibre, total protein, total fat, total sugars, amino nitrogen, soluble solids and soluble nitrogen. The protein quality of the tempeh samples were evaluated by growth of rats, PER and NPU of diets.

It was concluded that a Tempeh like product could be prepared successfully with a mixture of soybean and groundnut. Fermentation of soybeans or a mixture of soybean and groundnut resulted in an increase in the crude fibre, fat and soluble solids. No differences were observed in the growth rates of rats or PER or NPU when autoclaved unfermented soybeans and soybean Tempeh samples were fed. Tempeh prepared from a mixture of soybean and groundnut had a better protein quality than soy Tempeh. All the Tempeh samples tested had PER values above 2.0.

Soybeans are consumed as daily articles of food primarily in the oriental countries such as Japan, Korea, China and Indonesia[1–4]. The high protein and fat contents encourage its usage in diet. But the characteristic beany flavour and the anti-nutritional factors like the inhibitors are its drawbacks. Both the above defects are overcome in the microbial fermentation processes involved in the preparation of the Oriental soybean foods like the Tempeh, Ontjom, Tofu, Miso and Shoyu. Among the above foods Tempeh is a popular Indonesian food made from cooked dehulled soybeans by fermenting them with the Tempeh mold. Traditionally Tempeh is prepared with mixed cultures of *Rhizopus* of which *Rhizopus oligosporus* NRRL 2710 is a typical representative. The fermentation eliminates the beany flavour of raw soybeans and gives it a bland flavour. The process synthesizes desirable constituents such as vitamins, increases digestibility, changes the physical state and produces acceptable colour[4–5].

Several methods have been tried to standardise the processing of Tempeh. In the laboratory two important methods have been developed based on the domestic methods[1,2,5]. After fermentation, Tempeh has been found to have improved protein quality exhibited by increases in PER values over unfermented soybeans[6,7]. These have been attributed to better availability of amino acids liberated from the beans during fermentation[8] and to better digestibility of Tempeh due to increase in soluble solids and soluble nitrogen[9,10].

An attempt has been made to develop a new type of Tempeh namely Tempeh with a mixture of soybean and groundnut (1:1 on dry weight basis) using the three cultures of *Rhizopus oligosporus* namely NRRL 2710, 2549 and 514. The feasibility of processing such a product and its nutritional quality has been studied.

Materials and Methods

Raw materials: Soybeans used in all these experiments were of Bragg variety. The groundnuts used were purchased from commercial sources, partially defatted (without damaging its shape) and used for admixture with soybean for the preparation of Tempeh.

Cultures used: The cultures for the processing of Tempeh were *Rhizopus oligosporus* NRRL 2710, 2549 and 514. They were maintained on potato-dextrose-agar (PDA). They were obtained from Northern Regional Research Laboratory, Peoria, Illinois, U.S.A.

Preparation of Tempeh: Whole soybean was soaked in water (1:3), acidified with lactic acid (0.85 per cent), for 16 hr and was dehusked mechanically. Groundnut was soaked in water (1:3) for one hour. Equal parts of groundnut and soyabean (on dry weight basis) were mixed and cooked at 15 lb pressure for 20 min. The pH of the resultant cooked beans was found to be between 4.5 and 5.5 They were cooled to room temperature (25–28°C), poured into perforated steel trays (previously sterilized) and inoculated with the spore suspension of *Rhizopus oligosporus* (spore suspension was prepared

from a seven-day old sporulated culture of *Rhizopus oligosporus* grown on PDA in a Roux bottle). Thirty ml of sterile water was added to each Roux bottle for preparing the spore suspension. This was adequate for inoculating 500 g of the sample in a tray. The trays were kept for fermentation in a humidifier at 31°C for 20 to 24 hr at 50 per cent humidity. At the end of fermentation the samples were cut into small cubes, dipped in 10 per cent saline and dried in a cross-flow drier between 70° and 80°C. The dry Tempeh samples were sealed in polythene bags and stored at room temperature for future use.

Chemical analysis: The dry Tempeh samples were analysed for the following constituents: moisture, ash, crude fibre, total protein, total fat, total sugars, amino nitrogen, soluble solids and soluble nitrogen. All the samples were tested for the presence of trypsin inhibitor. All the Tempeh samples obtained were screened for the presence of aflatoxin (qualitatively) by the TLC method[12–14].

Growth rate and PER: PER (Protein Efficiency Ratio) was determined according to Chapman *et al.*[15]. Eighty weanling male rats of Wister strain weighing an average of 35 g were divided into eight groups. They were housed in wire screen bottom cages. Food was made into the form of a thick paste and given *ad libitum*. Water was supplied in polythene bottles. Diet was prepared at 10 per cent protein level with 2 per cent mineral supplement (Hubbel *et al.*[16]) and 1 per cent vitamin supplement (Chapman *et al*[15]). Diet was fed for four weeks. Body weights were recorded once in a week.

Net protein utilization (NPU) studies: The NPU was determined according to the NRC method[17]. The NPU values of Tempeh samples were determined from a 10-day experimentation. The strain *Rhizopus oligosporus* NRRL 2710 was chosen as the representative strain for the preparation of Tempeh samples used in this experimentation.

Results and Discussion

Table 1 gives the analysis of proximate composition of the processed Tempeh samples. There were no differences in the moisture and ash content of the samples studied. The crude fibre content of Tempeh samples prepared from a mixture of groundnut and soybean were higher than the unfermented control. This increase might be due to the contribution by the fungal mycelia or it could also be due to the increase in the soluble solids due to the action of the fungus. The soluble solids might have got leached out during the processing of the Tempeh.

The solubility of the solids of cooked soybean increased by the fermentation process when *R. oligosporus* 2549 and 514 were used. In the case of the mixture of groundnut and soybean the soluble solids increased from 13.0 to 25.0-29 per cent in the three strains of Tempeh samples used. This is comparable with the results of Van Veen and Schaefer[5] and Stainkraus *et al*[9]. Similarly the soluble nitrogen content of cooked soybean slightly increased on fermentation but in the case of Tempeh prepared with a mixture of soybean and groundnut the soluble nitrogen values rose from 0.90 to even 1.50g per cent. These changes might be responsible for the higher digestibility and biological values reported for Tempeh samples over soybean samples by Stainkraus *et al*[9,10].

There was a slight increase in the protein content of soybean Tempeh samples fermented by using *R. oligosporus* NRRL 2710. But no change was observed when the soybean Tempeh samples were prepared from *R. oligosporus* NRRL 2549 and 514. When a mixture of soybean and groundnut was used, protein content of Tempeh samples increased in the case of *R. oligosporus* NRRL 2710 and 514 but decreased when strain NRRL 2549 was used.

The fat content of both the types of Tempeh samples showed an increase over their control samples. In the

TABLE 1. PROXIMATE COMPOSITION† OF TEMPEH SAMPLES

Description of Tempeh	Fungal strain	Moisture	Ash	Crude fibre	Fat	Protein	Reducing sugars	Amino nitrogen	Soluble nitrogen	Soluble solids
Soybean	—	6.0	5.11	4.0	21.9	40.0	3.9	3.0	1.1	25.0
Soybean Tempeh	2710	5.8–6.0	4.5–5.2	3.7–3.9	20.7–26.4	42.8–43.5	5.1–5.2	2.6–3.9	0.9–1.2	24.0–26.0
Soybean Tempeh	2549	4.6–5.6	5.9–7.6	2.7–2.8	26.5–26.0	39.6–40.6	4.4–4.5	2.6–3.4	1.1–1.2	25.9–33.8
Soybean Tempeh	514	5.8–7.3	5.2–5.6	2.8–3.0	25.0–28.6	39.2–40.8	3.2–4.1	3.0–3.9	1.1–1.2	26.6–30.6
Groundnut**+ Soybean (1:1)	—	6.4	4.1	2.0	25.3	35.0	5.7	3.1	0.9	13.0
Groundnut**+ Soybean Tempeh	2710	6.2–6.9	3.8–4.3	2.7–3.0	25.8–27.6	35.7–36.7	5.0–5.3	2.2–3.2	0.9–1.0	28.0–29.0
„	2549	6.0–6.8	5.4–5.9	2.6–3.0	26.0–27.2	31.4–32.8	4.7–5.3	3.2–4.1	1.2–1.5	25.–026.4
„	514	6.3–7.2	4.0–6.0	2.9–3.0	24.6–28.7	36.1–36.5	3.5–4.8	2.1–3.2	0.9–1.2	27.0–29.0

† g/100g on dry weight basis; The values are from three replicates; **Rhizopus oligosporus;* ***Groundnut used was a partially defatted sample.

TABLE 2. PROTEIN EFFICIENCY RATIO OF DIFFERENT DIETS CONTAINING TEMPEH

Code No.	Source of protein	Body weight of rats (g.) Initial	Final	Increase	Food intake g.	PER (Body weight gains / protein consumed)
SM	Skim milk–Standard	35.2±04.9	116.6±4.49	81.4± 4.30	278± 9	2.91±0.080
SY	Soybean (Autoclaved, unfermented)–Control	35.2±0.52	90.4±2.21	55.2± 2.48	248±13	2.22±0.053
ST_1	Soybean Tempeh (2710)	35.2±0.51	89.7±1.08	54.5± 3.07	248± 8	2.18±0.071
ST_2	Soybean Tempeh (2549)	35.2±0.51	93.7±2.73	58.5± 2.58	254± 5	2.30±0.064
ST_3	Soybean Tempeh (514)	35.2±0.50	87.3±2.96	52.1± 2.66	245± 5	2.12±0.063
GST_4	Groundnut+Soybean Tempeh (2710)	35.3±0.58	108.8±3.30	73.5± 3.42	281±12	2.56±0.063
GST_5	Groundnut+Soybean Tempeh (2549)	35.2±0.40	114.6±2.00	79.4± 1.99	280± 4	2.84±0.090
GST_6	Groundnut+Soybean Tempeh (514)	35.3±0.52	101.7±2.29	66.4±83.58	273± 7	2.42±0.084

Test of significance

Source of variation	Degrees of freedom	Sum of squares	Mean squares	'F'	% significance
SM vs ST	1	2.05	2.05	22.8	0.01
SM vs GST	1	0.47	0.47	5.2	0.05
ST vs GST	1	0.83	0.83	9.2	0.01

Diet contains 10% protein and 10% fat.

case of soybean it was from 21.9 to 23.26 per cent and in the mixture of soybean and groundnut from 25.3 to 27.0 per cent on fermentation. The above observation is slightly at variance with the earlier reports[1–4] which indicate that there is either a fall or maintenance of fat levels during fermentation. No special trend could be noted in the case of the amino nitrogen content of the amples.

Presence of trypsin inhibitors and aflatoxin: Trypsin inhibitor and aflatoxin were determined according to Borchers *et al.*[13] and Pans *et al*[14]. Test for the presence of trypsin inhibitor in soybean (raw), soybean autoclaved unfermented and all the Tempeh samples showed that this was destroyed even by the autoclaving involved in the preparation of the samples. Hence neither the control nor the Tempeh samples showed any trypsin inhibitor activity. All the samples were found to be free of aflatoxin.

PER and NPU values of diets: The data on the growth of rats, PER and NPU are given in Tables 2 and 3. The PER value obtained with soybean (autoclaved, unfermented control) was 2.22, while the PER values obtained with soybean Tempeh diets ranged from 2.12 to 2.3. These values are in agreement with the values reported by different workers[10,11,18–22]. Tempeh prepared from a mixture of groundnut and soybean gave PER values ranging from 2.42 to 2.84 as against 2.91 obtained with skim milk powder. The food intake

TABLE 3. NPU VALUES OF TEMPEH* SAMPLES

Code No.	Source of protein	Body weight of rats (g.) Initial	Final	Increase	Food intake	NPU
SM	Skim Milk–Standard	64.6±1.14	98.4±3.13	33.8±2.49	114±5	72.8±3.94
SY	Soybean (Autoclaved, unfermented)–Control	64.5±2.12	92.5±1.42	28.0±1.92	119±5	56.7±2.40
ST	Soybean Tempeh (NRRL 2710)	64.5±1.39	98.8±2.91	34.3±2.15	123±4	58.7±3.20
GST	(Groundnut+Soybean) Tempeh (NRRL–2710)	64.4±1.45	101.1±4.46	36.7±2.00	128±1	65.8±3.09

Test of significance

Source of variation	Degrees of freedom	Sum of squares	Mean squares	'F'	% Significance
SM vs ST	1	1003	1003	23.3	0.01
SM vs GST	1	245	245	5.7	0.05
ST vs GST	1	259	259	6.0	0.05

**Rhizopus oligosporus* NRRL 2710 strain was used.

in the groundnut soybean Tempeh group is higher than the soybean Tempeh group. The PER value is also higher with respect to higher food intake. This increase in PER of groundnut—soybean Tempeh when compared to soybean Tempeh is significant at 1 per cent level.

The NPU values however are 59 and 66 for soybean Tempeh and groundnut and soybean Tempeh respectively. The increase in NPU has a marginal significance at 5 per cent level.

Studies on the sensory evaluation of the product have to be carried out.

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Studies on the Bottle Breakages, Number of Trips Made by a Bottle and Efficiency of Aluminium Caps Forming and Usage in a Small Sized Milk Processing Plant

K. N. S. SHARMA, M. R. SRINIVASAN AND R. C. NAGPAL
National Dairy Research Institute, Karnal

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Retail milk distribution in almost all the liquid milk plants in the country involve the use of standard glass bottles for filling and distribution. Formation of aluminium caps and their use as bottle cover are linked up operations. Breakage of bottles during handling and use of aluminium caps are important items in the cost of milk packaging and distribution. The present paper deals with the assessment of the percentage breakages of milk bottles (during washing, filling, storage and distribution) and wastage of metal foils and foil caps used as closure in a liquid milk plant handling about 5000 litres per day and filling and distributing 2,000 to 3,600 bottles per day. From the observations made over a period of 510 days, it is concluded that the average percentage breakage of bottles to be 1.00. The average number of trips to be expected of a bottle being about 80 and average wastage of aluminium caps 10 per cent.

Milk distribution in organised milk plants apart from ensuring quality milk in tamper-proof containers involves considerable cost of the bottled milk. Distribution cost which determines the consumer's price is a reflection of the efficiency of milk handling by the organisation. Efficient management depends on correct information on all aspects of costing of milk distribution. Inspite of the fact that standards are specified for the quality of glass bottles to be used in milk distribution, bottle breakage depends on the factors of washing, filling and handling operations during distribution. These factors have direct relation to the scale of milk handling, type of machinery used and skill of labour employed. There is scope for keeping the breakage to a minimum in a modern plant equipped with standard machinery and employing trained technicians, in spite of the factors contributing to bottle breakage. This task of the manager can be achieved if accurate information is made available on the various aspects of bottle usage and bottle capping.

Studies have been carried out in the West to fix the minimum breakages of bottles as well as to ascertain their number of trips. No published reports are available in India on these aspects. The present study was, therefore, undertaken to collect information on the breakage of bottles and use of aluminium foils in small size milk processing plants and to estimate the average number of trips per milk bottle.

Materials and Methods

The investigation was carried out at the experimental dairy of the National Dairy Research Institute, Karnal. The experimental dairy is a semi-commercial unit largely used for training and product development. It has a fully equipped liquid milk processing and bottling section. The dairy handles about 5,000 litres of milk per day. The number of half litre bottles handled per day vary from 2000 to 3600.

The milk is distributed by mobile van within the campus of the Institute once in the morning and once in the evening, covering a distance of 15 to 20 km each time. Approximately half the quantity of bottles are sold in the morning and the remaining half in the evening. The bottles are stacked in standard iron or plastic crates.

The study on the breakage of bottles and wastage of aluminium foils was conducted during a period of 510 days from 5th Feb. 1972 to 30th June 1973.

(*i*) *Breakage of bottles at different stages of handling:* The breakages during washing, filling and distribution were studied by a trained operator. He recorded (*i*) the number of bottles fed to bottle washer and the number that came out intact, (*ii*) number of bottles fed to bottle filler and number of bottles filled and kept in cold store, and (*iii*) number of bottles sent for distribution and number of bottles received intact in the dairy before washing. The differences in each case were taken as the number of bottles broken during washing, filling and distribution.

The percentage of breakage, during different stages of handling as well as the whole operations were calculated for each day and also for the entire period. The

frequency distributions of the daily percentage breakages were prepared. Correlation coefficients between the number of bottles handled and number of bottles broken were calculated for each stage of handling and also for the entire handling.

(*ii*) *Estimation of the average number of trips made by a bottle:* Two mathematical models were studied to estimate this viz., (*a*) constant per cent breakage model or static model, and (*b*) variable breakage model or dynamic model.

(*a*) *Constant percent breakage model:* In this model the overall percentage of breakage (p) observed during the period of study was considered as normal and constant and the complement of this $(1-p=q)$ as the proportion survived. Then,

$$Y_t = q\,Y_{t-1} \qquad (1)$$

where Y_t is the number of bottles survived on the t^{th} day, q is the percentage of survival. Y_{t-1} is the number of bottles survived on the $(t\text{-}1)^{th}$ day. The model assumes that (*i*) the proportion of bottles survived remains the same each day; (*ii*) the strain on bottle due to constant use has no effect on the survivability of the bottles; (*iii*) the bottles distributed on any day will be returned the next day; and (*iv*) the bottles broken by the customer has no effect on the survivability in the dairy as the same is replaced by the customer.

Starting with $Y_o = N$ bottles to begin with, the bottles survived on the first dayis qY_o, the second day q^2Y_o, etc. If the last bottle in this batch remains on the n^{th} day the total number of trips made by Y_t bottles will be

$$\Sigma Y_t = (q + q^2 + q^3 + \ldots\ldots\ldots\ldots\ldots + q^n)\,Y_o$$
$$= q\,\frac{1-q^n}{1-q}\,Y_o$$

and average number of trips will be

$$y = q \times \frac{1-q^n}{1-q}.$$

The value of n can be found out as

$$1 = q^n Y_o \text{ or } n = \frac{\log Y_o}{-\log q}.$$

As q is less than 1 and n is sufficiently large (500), q^n will almost be equal to zero. Hence the average number of trips made by the N bottles would be

$$y = \frac{q}{1-q} \qquad (2)$$
$$= \frac{\text{proportion survived}}{\text{proportion broken.}}$$

Once the average percentage breakage is estimated reliably, the average number of trippage could be ascertained with the help of this model.

(*b*) *Variable breakage or dynamic model:* In this model the number of bottles survived on any day is taken to be the function of the number of bottles survived the previous day, number of bottles broken and number of bottles added on the day, that is,

$$Y_t = Y_{t-1} + a_t - b_t \qquad (3)$$

Y_t = No. of bottles survived on the t^{th} day.

Y_{t-1} = No. of bottles survived on the $(t\text{-}1)^{th}$ day.

b_t = No. of bottles broken on the t^{th} day.

a_t = No. of bottles added on the t^{th} day.

If Y_o is the number of bottles taken to start with is reckoned as a_1, the number of bottles added on the first day, and the observations are taken for n consecutive days and if Y_{n+1} is the number of bottles left on the $(n+1)^{th}$ day, then the average number of trips made by a bottle during the period can be worked out to be

$$y = \frac{\Sigma^n Y_t}{t=1} - \frac{Y_{n+1}}{\Sigma^n a_t} \qquad (4)$$
$$t=1$$

The minimum number of days the observations have to be taken to estimate the number of trippage has been worked out.

(*iii*) *Estimation of losses in aluminium foils:* The aluminium foil roll used for cutting caps was weighed before and after the filling of milk in bottles upto 5 g accuracy. The quantity of scrap left over was also weighed upto 5 g accuracy. The number of caps that could be cut from the quantity of foil used was calculated by taking 2,800 caps per kilogramme of foil as recommended by the firm. The difference between the number of caps that could be cut and the number of bottles actually capped was taken as the avoidable losses in caps.

The percentage of scrap as well as the percentage of avoidable losses in caps were calculated for each day as well as for the entire period of study.

To ascertain the quantity of scrap and percentage of unavoidable losses in the use of aluminium foils, the actual quantity of aluminium foil required, and the scrap weight to cut 60 caps leaving the minimum space in between two caps was obtained. It was found that the weight of the aluminium foil was 20.1636 g and weight of the scrap was 6.0066 g and the percentage of unavoidable loss was 29.79. The average weight of a cap was 0.2360 g.

Results and Discussion

The average breakage of bottles per day during washing was observed to be 0.40 per cent with a range of 0 to 1.70 per cent; during filling 0.30 per cent with a range of 0.04 to 1.5 per cent; and during distribution 0.32 per cent with a range of 0.0 to 1.70 per cent (Table 1). The overall per cent breakage based on all the operations

TABLE 1. FREQUENCY DISTRIBUTION OF BREAKAGE OF BOTTLES DURING DIFFERENT OPERATIONS OF HANDLING

% Broken (range)	Broken during			Range (%)	Complete handling
	Washing	Filling	Distribution		
0.0–0.1	12	26	24	0.0–0.2	1
0.1–0.2	60	97	76	0.2–0.4	13
0.2–0.3	200	163	149	0.4–0.6	30
0.3–0.4	102	149	111	0.6–0.8	88
0.4–0.5	28	48	85	0.8–1.0	157
0.5–0.6	26	10	35	1.0–1.2	113
0.6–0.7	17	8	14	1.2–1.4	48
0.7–0.8	19	4	6	1.4–1.6	27
0.8–0.9	12	2	3	1.6–1.8	19
0.9–1.0	11	1	4	1.8–2.0	5
1.0–1.1	8	1	2	2.0–2.2	3
1.1–1.2	7	0	0	2.2–2.4	4
1.2–1.3	5	0	0	2.4–2.6	1
> 1.3	3	1	1	2.6–2.8	1
Total	510	510	510		510

was 1.02 with a range of 0.17 to 2.80 per cent. Though the range of variation in the percentage breakage was wide, it was observed that the percentage breakage was less than or equal to 0.4 per cent in 73.0 per cent days during washing and 85.3 per cent days during filling and less than or equal to 0.5 per cent in 87.2 per cent days during distribution (Table 1). Takin g only these observations, the average daily per cent breakage of bottles during washing, filling and distribution were worked out to be 0.23, 0.25 and 0.28 respectively.

For all the three operations taken together, the percentage breakage was less than or equal to 1.2 in 78.8 per cent days (Table 1). The average of these observations was observed to be 0.80 per cent. If sufficient care and caution is exercised during the three operations, there is considerable scope to bring down the percentage breakage from 1.02 to 0.80 per cent.

The use of variable control charts for fraction (or percentage) breakage (p. Charts; Grant, 1972) was made to ascertain whether higher percentage of breakage observed were within the normal variations or not. The observations during the first month of study was taken to set up the initial control charts. Observations during subsequent months were made to plot the upper and lower control limits. The daily observations were then plotted. It was observed that all the points were within the control limits. This revealed that though on some days high percentage breakage was observed, it was within the normal limits of variation.

The correlation coefficient between the number of bottles handled and number of bottles broken daily was observed to be 0.574 during washing; 0.379 during filling; 0.101 during distribution and 0.576 for all the three operations taken together. All the correlation coefficients were highly significant ($P<0.01$) except during distribution where it was significant at 5 per cent level. The very low correlation coefficient during filling and distribution suggests that the number of bottles broken is not influenced by the total number of bottles handled. It was observed that on 95 per cent of the days the number of bottles broken was less than or equal to 15 in each of the operations-filling and distribution, while in washing it was so on 82 per cent of the days.

Estimation of number of trippage: The average daily breakage of bottles due to handling was observed to be 1.02 per cent with sufficient scope to reduce it. With the existing efficiency, the normal breakage can be reckoned as 1 per cent in the experimental dairy. This gives us the number of trippage to be 99 from the first model.

The number of trippage of bottles from model 2 on the basis of 510 observations was 81. To ascertain the number of days for which the observations are to be made to get consistant estimates of the number of trips, the trippages were calculated by taking first 3 months observations and subsequently at the end of every month. The results are shown in Fig. 1.

It can be observed that the number of trips increased upto 11 months or 334 observations and later on the number of trips were almost constant. This suggests that the observations on the breakage and addition of bottles should be maintained at least for one year to estimate the number of trips made by a bottle.

The number of trips ranged from 78 to 81 for observations from 11 months or more. This suggests that the average number of trips made by a bottle according to model 2 can be taken as 80.

The model 1 gives the number of trips made by a

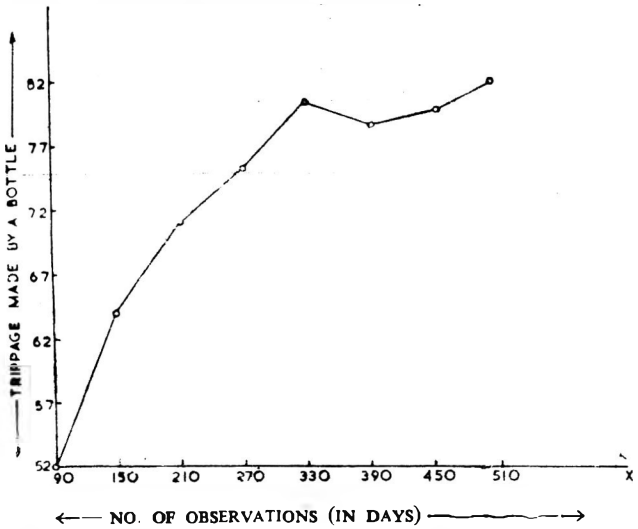

Fig. 1. Number of trips in relation to number of observations

bottle as 99 while model 2 gives the same as 80. The second model is a dynamic model which takes into account the daily breakage and also for after months observations or more the estimate of number of trips made converges to a single point. The estimate obtained from this model, therefore, appears to be more reliable. The first model on the other hand exaggerates the number of trips.

The cost of bottle per trip was worked out to be 0.81 paise taking the purchase price of 65 paise during the period of study.

Estimation of wastage in aluminium foils: The average percentage of aluminium foil thrown as scrap was worked out to be 27.00 with a range of 14 to 43. This was based on 506 observations. On 4.9 per cent days the loss in aluminium foil as scrap was less than 21 per cent while on 4.7 per cent days the loss was above 31 per cent. In the remaining 90.4 per cent days the loss was between 21 to 31 per cent. It was observed that the average loss in aluminium foil as scrap was within the normal limits as recommended by the manufacturers to get optimum efficiency.

The average avoidable loss was 10.07 per cent with a range of 0 to 40 per cent. A little over 75 per cent of the observations showed an avoidable loss of 14 per cent or less.

The cost of foil during the course of investigation was Rs. 10.75 per kg. This gave the cost of capping 100 bottles to be 41.6 paise. If the value of the scrap is reckoned as half the price of foil (i.e. Rs. 5 per kg), this cost would be 36.4 paise.

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The authors (S. R. Shurpalekar and K. V. L. Venkatesh) of the paper entitled "Spreader Roller Device for Objective Evaluvation of Rolling Characteristics of Papad Dough" (*J. Fd Sci. Technol.*, 1975, **12** (3), 14.) wish to inform that Fig. 1 is the Spreader Roller Apparatus Developed by the Packaging Discipline of CFTRI, Mysore.

RESEARCH NOTES

A CIRCULAR PAPER TECHNIQUE FOR STUDY OF MICROBIAL GROWTH

A simple, inexpensive sectored paper chromatographic method to study the growth of organisms (*A. niger* and *P. digitatum*) associated with citrus fruits and the response to chemicals (thiabenzadole) is described.

In microbiological studies it is necessary to observe multiplication, sporulation and response to chemicals of different fungi. This is especially so when the nature of microbial contamination and its response to fruit surface treatment are to be investigated. In the conventional methods, culture media are used for study of growth and sporulation. Kilpatrick[1] reported a simple procedure for induced sporulation of fungi using a filter paper technique. He demonstrated that sporulation of fungi without plant tissue could be successfully induced when agar plugs containing hyphae were transferred to a previously saturated non-sterile filter paper.

In our laboratory, work is in progress to study the growth of two organisms viz., *Aspergillus niger* and *Penicillium digitatum* commonly associated with citrus fruits and their responses to various chemicals. In order to assess the efficacy of a few chemicals a more expeditious and simple procedure was desired. Agar culture technique is not only expensive but time consuming. Besides, the effect of a chemical as such on the fungal characteristic would not be obtained by Kilpatrick's method. A modification developed in our laboratory was found to be more satisfactory for purpose of this study. The procedure described below offers a quicker method to study fungal growth responses within a period of 2 to 4 days. The method is as follows: A clean desiccator is first disinfected by alcohol and greased properly to obtain air tight condition. A petridish containing distilled water is kept in the bottom chamber of the desiccator to provide humid atmosphere. Within 24 hr such humid condition is established. A filter paper with sectored circular partitions was employed for the growth of the organisms. Ranjan[2] reported that a sectored circular paper used in the chromatographic technique showed no interference from one sector to another although a single solvent solution is fed through a central wick to all the sectors simultaneously.

In order to simulate the natural substrate conditions as obtaining in the peel of the citrus fruits, water extract of healthy citrus peel was used. The extract was sterilized with propylene oxide as suggested by Klarman[3]. A petridish containing sterilized citrus fruit juice was kept on perforated china clay plate in the desiccator. A filter paper wick was fixed at the center of the filter paper and, thus, the circular paper remained suspended in the air. Feeding of nutrient solution was obtained in all directions through the wick. Small agar plugs containing hyphae of *Aspergillus niger* removed from a 7 day old culture growing on potato-dextrose-agar, were transferred to the filter paper at a spot 2 cm away from the position of the central wick and incubated at room temperature (24±2.5°C). Within 3 to 5 days the hyphae grew from the plug on to the surface of the paper and spored abundantly (Fig. 1 and 2.) When response of chemical(s) is to be studied, two concentric circles are drawn on the filter paper. On the sector of the inside circle, solution of the chemical is spotted and the concentration of chemical is increased, if necessary, by frequent applications with intermitant drying as is usually done in the chromatographic technique. An agar plug is placed on a spot in the same radial direction on the outer circle sector.

In order to verify the efficacy of this procedure the effect of three concentrations of thiabenzadole (TBZ) on the growth of micro-organism was studied. As may be seen in the Table 1, this chemical was effective at concentration of 2000 ppm for *A. niger* and 1000 ppm for *P. digitatum*. Lower concentrations of this chemical were not as effective (Table 1). Thus in a single trial with sectored circular paper it was possible to have sufficiently

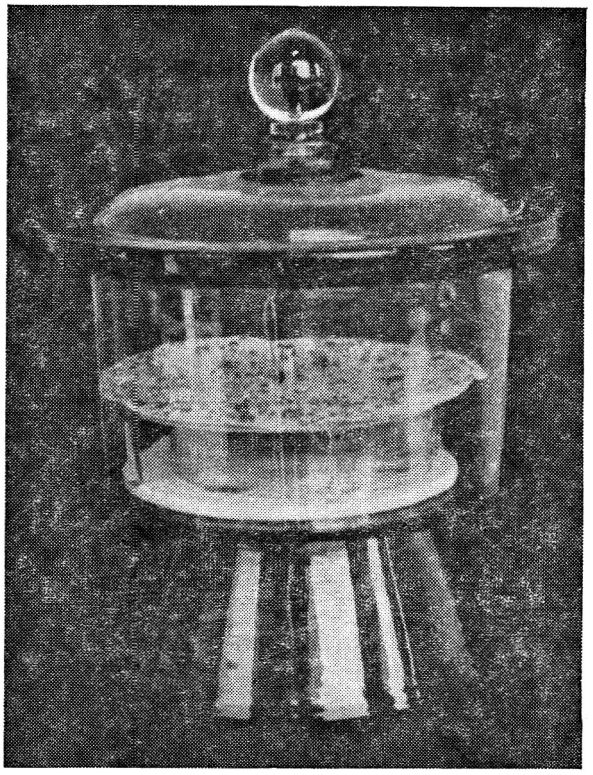

Fig. 1. Circular paper assembly in a desiccator

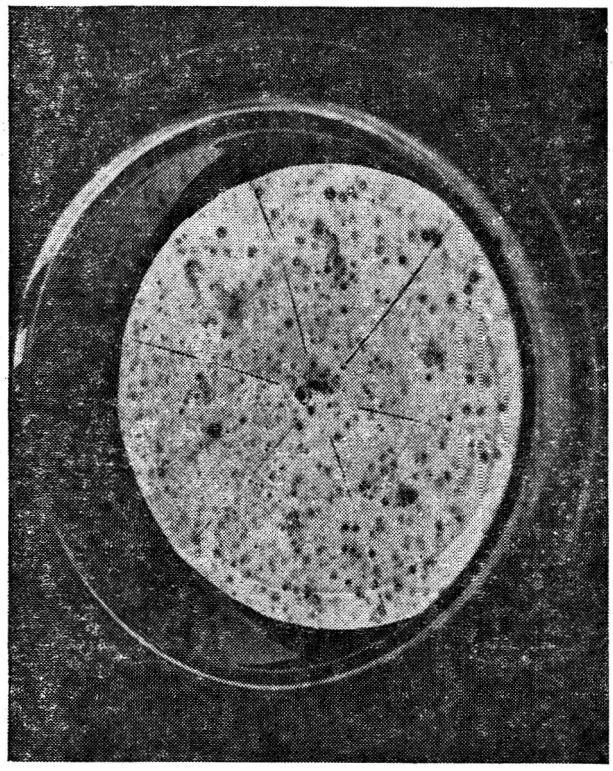

Fig. 2. Circular paper showing development of *Aspergillus niger*

good idea of the efficacy of the chemical in relation to organism to be studied.

The method is simple and inexpensive as it offers supply of nutrient at low level for the growth response of fungi. In our preliminary trials quick or abundant growth of fungi was obtained within 2 days with a nutrient solution. The method is useful not only for routine purposes but can be used in identifying unknown organism associated with the spoilage of fruits. This method, thus, offers a quick procedure of comparison of different fruit juices i.e. (different fruit surfaces) towards one or more organisms.

TABLE 1. GROWTH RESPONSE OF TWO ORGANISMS TO DIFFERENT CONCENTRATIONS OF THIABENZADOLE (TBZ)

A. niger growth/day	1st sector Control (no treat)	2nd sector 500 ppm	3rd sector 1000 ppm	4th sector 2000 ppm
1st day	—	—	—	—
2nd day	+	—	—	—
3rd day	+++	++	++	—
5th day	+++	+++	+++	—
P. digitatum growth/day	1st sector Control (no treat)	2nd sector 500 ppm	3rd sector 700 ppm	4th sector 1000 ppm
1st day	—	—	—	—
2nd day	+	—	—	—
3rd day	+++	+	+	—
5th day	+++	+	+	—

(—) No growth (+) Growth present
(++) Moderate gorwth (+++) Profuse growth.

Department of Biochemical Engineering & Food Technology, H. B. Technological Institute Kanpur.

KM. REWA ARORA
W. B. DATE

4 *January* 1975.

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CLEAN-UP OF CEREAL AND MILK FOR DETERMINATION OF RESIDUES OF ORGANOCHLORINE INSECTICIDES

A simple procedure for the clean-up of cereal and milk samples upto 5 g on a basic alumina plate, suitable for subsequent determination of organochlorine insecticide residues by thin layer chromatography is described.

Cereals form 30 per cent of the balanced diet of an average Indian. A large number of different types of insecticides are used for protecting cereal crops of which the organochlorine insecticides occupy an important place. There are reports of these being mixed directly with the grains by traders[1] to prevent infestation. Such indiscriminate uses have resulted in high levels of residue in some cases.

In India, analysis of several samples of milk reveal the presence of organochlorine insecticide residues in them. The feeding of cattle with the hay of cereal crops and the drift of insecticides during application to crops near grazing grounds, are the factors responsible for this. Reports of the presence of organochlorine insecticide in milk have originated from different areas of the world like Denmark[2], France[3], Germany[4], Poland[5] and U.S.A.[6].

One of the recommended methods for the determination of organochlorine insecticide residues in cereals is to extract with hexane in a Soxhlet extractor and clean-up by partitioning into dimethylformamide and eluting through an alumina column[7] prior to determination by thin-layer, paper or gas-liquid chromatography. Another method, followed by the United States Food and Drug Administration[8], is to extract with acetonitrile, clean-up on a Florisil column and determination by gas chromatography.

According to Mills' method[9] the fat is separated from the milk by centrifugation, and taken up in petroleum ether. The pesticides are then partitioned into acetonitrile and cleaned over a Florisil column. The cleaned extracts are injected into gas chromatograph for detection and estimation of residues. In another method, milk is extracted with acetone-hexane mixture, the extract clarified by centrifugation, separated, the hexane

phase dried, its volume adjusted and then treated by the DMF partition process. The final extract requires treatment with an activated alumina column and is then suitable for either gas or paper chromatographic examination.

Screening of samples of foodstuffs for organochlorine insecticide residues is being carried out at the Central Plant Protection Training Institute, Hyderabad. Thin-layer chromatography has been used for the identification and determination of the insecticides. During the course of the work it has been found that the maximum quantity of sample that could be tolerated on a TLC plate was about 0.5 g, both in the case of cereal and milk samples, when the procedure of de Faubert Maunder[7] making use of DMF clean-up was adopted. The other recommended procedure utilising 'Florisil' (a synthetic silicate manufactured by M/s. John Mansville & Co., Florida) could not be used as the adsorbent is not available in India. Therefore a simple alternate clean-up method for cereal and milk samples has been worked out making use of a basic alumina TLC plate for clean-up. When this method is adopted it is possible to spot an extract equivalent to 5 g of the sample without any streaking, and it is described below.

The samples of cereals and milk were collected from different retail market vendors.

(*a*) *Extraction method for cereals:* Twentyfive g of the powdered cereal is moistened with 8 ml of water and shaken mechanically with 75 ml of a 3:1 hexane-acetone (v/v) mixture for one hour. An aliquot of the extract equivalent to 5 g is taken for clean-up.

(*b*) *Extraction method for milk:* Ten g of milk is shaken up with 1 ml of saturated sodium oxalate, followed successively by 25 ml each of ethyl alcohol, ethylether and petroleum ether, in a 125 ml separating funnel. The aqueous layer is discarded and the solvent layer collected in a 100 ml beaker and volume reduced to about 5 ml. This is quantitatively transferred to a 125 ml separating funnel, rinsed with petroleum ether and made up the total volume to about 25 ml. The insecticides in the extract are then partitioned to acetonitrile saturated with petroleum ether, by 3 washings of 10 ml, and collecting the acetonitrile in a second separating funnel. The combined acetonitrile is washed with 10 ml of petroleum ether saturated with acetonitrile and transferred to a 500 ml separating funnel containing 250 ml water and 50 ml saturated sodium chloride. The petroleum ether in the second separating funnel is washed with 10 ml of acetonitrile which is also added to the water. The water acetonitrile mixture is shaken well for 2 min and set aside for 30 min for the layers to separate. The water is drawn and discarded, and the petroleum ether is transferred into a stoppered measuring cylinder, through a plug of anhydrous sodium sulphate. The volume is made upto 10 ml so that 1 ml is equivalent to 1 g. Five ml of this is taken for clean-up.

Clean-up: The clean-up of the cereal and milk extracts is done by thin-layer chromatography, over a 500 micron thick basic alumina plate, using acetonitrile as the developing solvent.

(*i*) *Preparation of alumina plates:* Basic alumina supplied by the National Chemical Laboratory, Poona, is blended with calcium sulphate in the proportion of 9:1 by weight. Thirty g of this mixture is taken in a 100 ml stoppered conical flask and made into a slurry by shaking with 30 ml water for 2 min. The slurry is poured into a moving type of applicator, stationed on the first of the plates to be coated, and drawn over them. Four plates of 18×11 cm can be coated with the above quantity. The plates are air dried and then activated at 110°C for one hour and stored in a desiccator.

(*ii*) *Spotting:* The extract, equivalent to 5 g of the sample is taken in a 10 ml beaker and volume reduced to about 0.5 ml. This is spotted on the activated plate as a 2 cm band, across the width of the plate, 2 cm from the bottom, with the help of a 2 ml glass syringe with a Luer tip and a 27 gauge needle. A sketch of the spotted plate is given in Fig. 1.

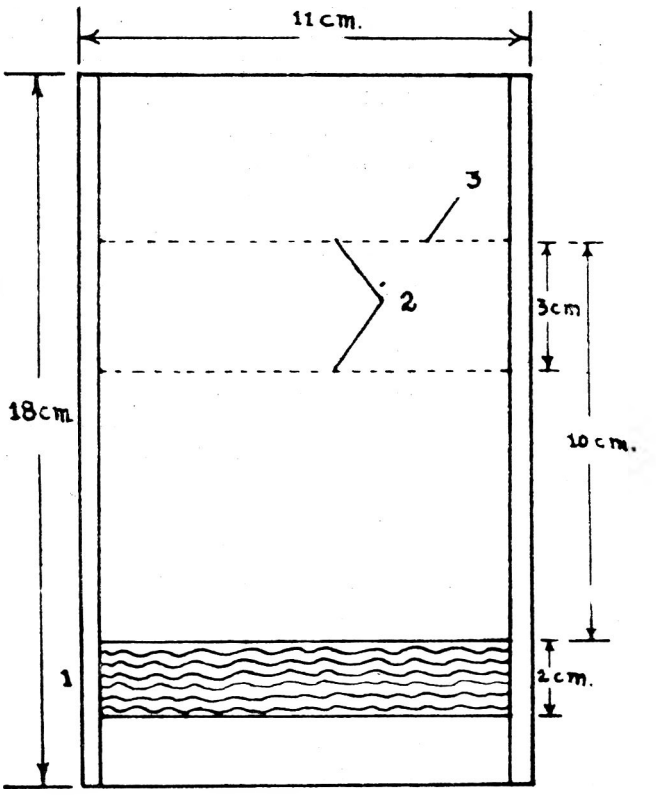

FIG. 1. Alumina-thin layer plate

1. Sample applied as a 2 cm wide band
2. 3 cm wide strip to be scraped out after development.
3. Solvent front.

(*iii*) *Development:* The spotted plate is developed in an airtight chamber in acetonitrile to a height of 10 cm. The developed plate is removed and the alumina layer to a width of 3 cm just below the liquid front is scraped out and collected in a 10 ml beaker. This alumina is extracted with 5×1 ml portions of acetone and filtered into a 10 ml beaker.

(*c*) *Detection and estimation of residues:* The cleaned extract is reduced in volume and spotted on a silica gel-silver nitrate thin-layer plate prepared according to Moats[10] along with spots of different standard insecticides, and developed in cyclohexane. The developed plate is air dried and exposed to ultra violet light (250 μm wave length) for 10 min. Pesticides appear as brownish-black spots on a light white background. Visibility of the spots can be improved considerably by exposing the plate to steam and irradiating for another 2 min. The lowest quantity that can be detected is 0.1 μg of DDT, endrin and lindane and 2 μg of BHC.

(*d*) *Recovery experiments:* Jowar, rice and wheat were fortified at 2 ppm levels with BHC, DDT, endrin and lindane and taken through all the above steps. The recovery of the added insecticides was 65 per cent in the case BHC and lindane and 90 per cent for DDT and endrin. Milk samples were extracted and cleaned up according to the above method, after adding insecticides at 2 ppm level. The recovery of insecticides was very good, ranging from 80 per cent for BHC, endrin and lindane to 95 per cent for DDT.

The recovery experiments with fortified samples revealed that the method is very efficient to clean-up extracts containing lipids, prior to determination of organochlorine insecticides by thin-layer chromatography. The chemicals and equipment required are available indigenously and are cheap. The TLC method followed by Morley and Chiba[11] can also visualise 0.1 μg of the insecticides, but the size of the sample is limited to 0.5 g of wheat giving a sensitivity of 0.2 ppm only. The screening method of Abbot *et al.*[12] using alumina coated thin-layer plates can tolerate only 0.1 g of the sample. Even though gas chromatography with electron capture detector is highly sensitive and can detect nanograms of the insecticides in samples, the size of the sample is limited to 100 to 200 mg. This gives a sensitivity range of 0.01 to 0.02 ppm, when 1 nanogram is quantitated. By using the clean-up method described above 0.1 μg of insecticides can be detected in 5 g of the cereal and milk giving the method the same sensitivity of gas-liquid chromatography with electron capture detector, without the need of sophisticated equipment and costly clean-up procedures. Further the method can be followed with a minimum period of training, and hence will be very beneficial to laboratories monitoring insecticide residues in these vital items of food.

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Central Plant Protection
Training Institute,
Hyderabad-500 030
20 *February* 1975.

V. LAKSHMINARAYANA
P. KRISHNA MENON

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ESTIMATION OF DIMETHOATE IN FOODS

A rapid and simple method for qualitative detection and quantitative estimation of dimethoate pesticide residue on foodstuff by solvent extraction, thin layer chromatography and colorimetry is reported. The yellow spots of the concentrated benzene extract, obtained on the TLC plate after spraying with palladium chloride is scraped into a test tube and eluted with N-N-dimethyl formamide and the absorbance of the clear solution, is measured spectrophotometricaily at the wave length of 435 nm.

Dimethoate [O–O–Dimethyl S–(N–methyl–carbamoyl methyl) phosphorodithioate], ($C_5H_{12}NO_3PS_2$) belongs to organic phosphate insecticide group, having colourless transparent crystal appearance with characteristic odour. It is a systemic and contact insecticide

and acaricide, used mainly against fruit flies, in particular the olive fly and the cherry fly[1]. It is also commonly being used against pests of vegetables. Dimethoate is known to control more than 200 injurious species of insects on over 100 crops and ornamental plants and thus, has a potential of being accepted as one of the most promising systemic insecticides.

The residues of dimethoate in the soils[2] and in sorghum forage[3] were studied. The bioassay study[4] on the persistence of dimethoate on cabbage and a few other investigations on dimethoate residues[5–8] are reported. The maximum tolerance limit advocated for dimethoate is 0.02 mg/kg body wt as acceptable daily intake[9]. With these considerations in view, a TLC method for its estimation in foods in general has been attempted in the present study.

Several sets of experiments are carried out to find out the most suitable agent for the extraction of dimethoate from foodstuffs (vegetables and cereals). The materials used are pulse (pea dal), *atta*, gourd, potato and green leaves. The results tabulated below show that all the foods exhibited more or less the same extractibility for added dimethoate with different solvents. The results are shown as averages from the items with each solvent.

Extracting agent	*Dimethoate recovered* (%)
Benzene	100
Chloroform	80
Acetone	75
Hexane	60
Petroleum ether	60
Acetonitrile	00
Solvent ether-hexane (2:1)	50

Finely chopped and well mixed representative sample (about 100 g) is taken in a blender and 200 ml of benzene is added and blended for 30 min. The process is repeated with another 100 ml of solvent. Then the filtration is carried out by Whatman No. 4 filter paper. The solvent in the filtrate is evaporated on water bath with care with a Kuderna Danish Evaporator, so that there is no loss of pesticide. The volume is then made up by adding quantitative amount of acetone before spotting on TLC plate. Fortunately purification of the extract or clean up[10] is not required.

Preparation of TLC plate: Glass plates of size 10×20 cm are coated with a slurry of silica gel G in distilled water (silica gel G 30 + water 60 ml) to a thickness of 250 μ. The plates are dried at room temperature for 20 min, then activated at 100°C in an air oven for 1 hr, and stored in a desiccator. The mobile solvent used was benzene and acetone (9:1)[11]. Chromogenic agent used was 0.5 per cent palladium chloride in 8 per cent HCl.

Preparation of standard graph and simultaneous estimation of residue in the sample: A 0.1 per cent standard solution of dimethoate (Tata-fison, purity 95 per cent) is prepared with acetone. A thin layer plate is spotted with different volumes *viz.* 10, 20, 30, 40 μl of standard solution along with the 50 μl of the sample solution (obtained by extracting the sample with benzene as previously). The sample is applied about 2 cm from the bottom edge of the plate. It is important to restrict the diameter of spot to 5 or 6 mm[11].

The plate is then allowed to be irrigated, letting the solvent front move upto 15 cm from the spotting point. It is then kept at room temperature for some time to evaporate the solvent completely and sprayed with the chromogenic reagent. The plate is then allowed to dry at room temperature just to evaporate the water of the chromogenic agent.

The spots are scraped (with the sharp edge of a knife) and taken in 5 ml (in each case) of N–N–dimethyl formamide in separate test tubes. The tubes are shaken occasionally for 5–10 min for the complete extraction of colour from silica gel. The contents of the tubes are centrifuged to remove silica gel. Then the optical densities of the clear supernatant liquid solutions are measured in the spectrophotometer at the wave length of 435 nm. Spectra of similar solutions of dimethoate have previously been checked to have a maximum near about this wave length. The concentration of the pesticide is obtained from the straight line obtained by plotting absorbance against the known concentrations.

The common trend in extraction cum clean up operations of pesticide analysis is to transfer the pesticide to a low boiling cheap solvent, light petroleum, after preliminary extraction using methyl cyanide[12]: (or isopropanol–petroleum mixture). The latter has been devised to replace the costly solvent, acetonitrile[13]. However, in this case dimethoate cannot be transferred to petroleum ether, after addition of water, because of its appreciable solubility in polar solvents. This necessitates evaporation of solvents which are unsuitable as they have high boiling points and are costly. The most efficient extractant in the single solvent extraction procedure here is benzene; its high boiling temperature (79–81°C) does not inconvenience, as dimethoate is affected only at more elevated temperature[14].

The colour developed on the chromatogram is amenable to direct reading in a photoelectric densitometer[15]. However, this is obviated in this study for two reasons—graining (or precipitation) which sometimes occur lending heterogeniety in the spot and, secondly,

the common densitometers do not have selective wave lengths or even filters.

Central Food Laboratory
Calcutta-16
15 *July* 1974

J. CHAKRABORTI
G. MUKHERJEE (MRS.)
A. K. MUKHERJEE
B. R. ROY

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BOOK REVIEWS

Indian Dairy Products: by K. S. Rangappa and K. T. Achaya, Asia Publishing House, Bombay, pp; 386, Price: Rs. 40.

The Chemistry and Manufacture of Indian Dairy Products, by the same authors was first published in 1948. It was an interesting review of development in dairy industries and covered a number of aspects of Indian dairying at that time. The book under review is a completely revised edition of this book and in bringing out this book, the authors have added new material which has accumulated since the last publication. The book is divided into three parts and deals with milk and unfermented milk products, fermented milk products and ghee.

In the first part the authors have described the major milch breeds of the country, their performance and production. The composition of milk and its variation due to various factors like breeds, season, feed and health of the animal are documented. Milk hygiene and the bacteriology of milk are dealt with in detail. Adulteration of milk and methods for its detection have been described. The second part deals with fermented milk products like *dahi* and butter. *Dahi* is liked throughout India and is consumed in large quantities unlike yoghurt which is its counterpart in other countries and is consumed only in small quantity. Very often the only milk protein which is available to a large section of the people is through *dahi*. As such it has a unique place in the Indian dietary. The authors realising this have devoted considerable part of the book to a detailed description of the preparation of *dahi*, its composition and nutritive value. As they have rightly pointed out a modernised *dahi* industry can provide a healthy, standard product of high nutritive value to a very large consuming public in Indian cities, making fuller utilisation of all the constituents of the available milk. Butter is another product which has a high economic value in the dairy industry. It is estimated that 46% of the total milk produced in India is used for making butter. In Indian villages large quantities of milk are converted into *dahi* butter churned and the butter milk either consumed as such or wasted. In the Chapters on butter, the authors have reviewed the indigenous methods of making butter and have shown defects and short comings in such a process. They have discussed the improvements which could be brought about in making butter by following slightly improved methods. They have rightly emphasised that radical changes cannot be introduced overnight in a cottage industry where the petty farmer produces small quantities of butter in his house for sale. The existing practices have to be improved to the benefit of both the producers and the consumers by affecting alterations wherever they are needed.

Nearly half the book is dedicated to the last part i.e. ghee. Ghee is the most important milk product in India. Its value is much more than of the other milk products. It is only right that the authors should place so much importance on this product. In the introduction, the authors have discussed ghee as a source of nutrients, the vitamin content, the fatty acid composition and the loss of the nutrients in the course of the preparation of ghee. In the next section various processes of preparation of ghee and the factors in processing which affect the quality of ghee are described in detail. The characteristics of good ghee, the flavour, the aroma, the colour and the granuality are described. The constitutions of ghee i.e., the fatty acids, the glycerides, the vitamins and trace elements are discussed. The variation of these due to several factors are described. The analytical characters like the Reichert value and the variation of these due to different causes have been described. It is difficult to find a more detailed account of the adulteration of ghee and its detection elsewhere than what is contained in this book. The subject has been treated in a masterly way discussing the methods that have been adopted for the detection of adulteration in ghee, the draw-backs of each method and the good points in it. A large amount of work has been done in this field. But there are not many text books which have attempted to bring all the results of the scientific research and discuss it in an objective and well coordinated way. The authors have done this to a great degree of success. The last chapter is about the defects in ghee, i.e., rancidity mostly caused by oxidative changes. Methods of measuring rancidity have been described.

The book is based to a large extent on published papers by Indian research workers in the dairying field. The reference to the papers have been given in detail. One signal service this book has done to the research workers in dairy industry is to provide excellent review of the work that has already been carried out and point out areas where research work is needed. Indian dairy industry has far too long been imitating dairy industry abroad. Some of the dairy products on which considerable research has been done like cheese do not have a sufficient market in India. But other products on which research is lacking like *dahi*, *panneer* and other indigenous dairy products have a wide market. The book tries to set a balance between these two and show the possibilities of research work concentrating more on process and methods which are needed in the country. Our dairy industry is based on the production of farmers with one cow or two cows or buffaloes with as little as two to ten litres of milk per day. It is not the same in other

countries where a dairy farmer produces a few hundred litres or more of milk. The research work that is needed in the country is to help the individual farmer carrying on his trade with one or two cows under conditions which will not be tolerated in other prosperous dairying countries. It is in this locale that the authors of this book have tried to give suggestions for improvement, modification and reorganisation. To that extent the book is a very useful contribution to the dairy industry in India.

Indian dairying is on the brink of a break-through and in the next few years it is anticipated that several new milk products will be on the market. Milk powder of different compositions, infant foods, ice-cream of different composition, yoghurt powders, milk substitutes, butter substitutes, etc. It would have been appropriate if the authors had paid more attention to these products which are bound to flood the markets in the future. Also the chapter on the Indian milk sweets industry could have been expanded.

Though the book has been published in 1974, it is unfortunate that some of the research papers quoted are out dated. The amount of material that has been collected for bringing about this book, possibly could have been made a little more up-to-date by bringing in research work of a later period omiting some of the references to the earlier ones. Though this has not reduced the value of the book to the Indian dairy man, the reviewer feels compelled to make this remark in the hope that in a new edition the authors will take note of it. The authors have mentioned in the preface that "it is unlikely that they will engage themselves in a further edition of the volume", but the popularity of the present edition will surely encourage them to revise and bring out a third edition.

The publishers have to be congratulated on the nice get-up of the volume. The reviewer could not find any errors in printing. The book has met a long felt need for a compendium of research work in dairying and as the authors have rightly pointed out this is a necessary text-book for students in Dairying and Agricultural Science. No research worker in the field of dairying and allied sciences could afford to do without a copy of this excellent book. Likewise the man in the dairy industry. The price of the book is attractive for a volume containing so much information.

M. R. CHANDRASEKHARA

The Industrial Manufacture of Cassava Products: An Economic Study. by D. Edwards, TPI Report G88, Tropical Products Institute, 56/62 Gray's Inn Road, London WC1 X 8LU, 1974, pp. 43.

This booklet is one in the series published by Tropical Products Institute and covers studies relating to economic feasibility of production or processing ventures in different countries especially the developing ones. As cassava (tapioca) forms one of the high yielding tuber crops in many developing countries of Africa and South and South-East Asia, an authentic description of the manufacturing processes for production of chips, pellets and starch along with costing models for different capacity manufacturing units will not only serve as guidelines for the interested entrepreneurs, but is likely to generate new entrepreneurship in developing regions. As the cost models have been worked out on the basis of situation prevailing in the developing countries, they will be of great help to those involved or interested in cassava processing.

S. R. SHURPALEKAR

Triticale: Proceedings of an international symposium, El Batan, Mexico, 1-3 October, 1973, edited by: Reginald MacIntyre/Marilyn Campbell (IDRC-024e), International Development Research Centre, Ottawa, Canada K1G 3H9, 1974/pp. 251.

A decade's (1964–73) achievements of plant breeders, agronomists, geneticists, nutritionists, etc. form the content of this book on Proceedings of an International Symposium on this new man-made cereal (cross of wheat and rye). A multi-disciplinary approach of nearly 100 participants brought together on a common platform by International Development Research Centre has made possible, unique interchange of ideas. This will no doubt initiate newer interests and more vigorous action to overcome the existing problems relating to triticale development.

An exhaustive historical review on the development of triticale included at the beginning, will be of great use to different research workers interested in this field. Different research programmes on the development of triticale in Western and Eastern Europe, Germany, United Kingdom, Canada, U.S.A. and CIMMYT (Mexico) are reported in earlier papers of the Proceedings. These are followed by the details of new programmes of work initiated in developing countries like India, Iran, Ethiopia, Algeria, Kenya, Chile, etc. Various challenging problems facing the breeder, agronomist, etc. form the last part of the book.

In view of triticale's outyielding potential when compared to wheat and also its adaptability to soils unsuitable for wheat cultivation and as nutritionally it has been found equally good or better than other cereals including wheat, triticale is probably the most promising

answer to hungry world's quest for more food. This is all the more so, as the Proceedings have highlighted the possibility of triticale pushing forward the frontiers of today's agricultural area into colder, hotter, or drier regions of the world, so that the total cultivated area increases significantly.

As the Proceedings cover a wide spectrum of different lines of work on triticale, this book is an important addition to the library of agricultural scientists as well as cereal technologists.

S. R. SHURPALEKAR

Fish and Shellfish Hygiene: Report of a WHO Expert Committee convened in cooperation with FAO/ WHO, 1211, Geneva, 27, Switzerland, 1974, pp. 62, Price Sw. fr. 6.

Hygiene in fish and shellfish handling, except in case of material meant for export, is conspicuous by its absence in India and possibly in other developing countries as well. Reporting, recording and investigating of fish-borne diseases are all irregular and scanty rather than systematic and comprehensive. There was a report just the other day (Indian Express, March 8, 1975) on how fisherwomen in coastal Karnataka who handled mackerel transmitted *E. coli* contamination to their infants causing severe gastroenteritis. Preventive work would not normally be initiated on the basis of such stray reports.

Adoption of hygienic practices in fish and shellfish handling through the steps of catching, transport and marketing would need capital investment. There is now neither any incentive, obligation nor awareness to take to hygienic practices.

Against this background it is the duty of microbiologists, research workers and technologists dealing with fish handling and processing to create the necessary awareness in the public mind, among the municipal and public health workers and administrators on how fish and shellfish are potential sources of hazard to public health. It is to such technical personnel, in their endeavours to promote hygienic practices in handling of fish and shellfish, that the present book should serve as a guide and starting point.

In a relatively short space the book scans a wide horizon. Starting from environmental factors in relation to fish and shellfish hygiene, it classifies the principal human diseases resulting from ingestion or contact with fish and shellfish, the principal diseases of aquatic food animals as related to public health, and biotoxins of marine fish and shellfish. The book then gives guidelines for epidemiological investigations, for safe handling of fish, shellfish and their products and for inspection services. It spells out the requirements of training and education and offers recommendations related to programme development, and research and technology. The two annexes on recognised fish–and shellfish-borne diseases of man and on selected bibliography on fish and shellfish hygiene are valuable.

The book deserves to be widely taken note of not merely as a source of information but as a starting point for preventive hygienic and sanitary practices in fish and shellfish handling, distribution, marketing and processing.

N. V. SRIPATHY

Text Book of Meat Hygiene: by Horace Thornton and J. F. Gracey, Bailliere Tindall Henriella Street, London WC2E 8QE, 6th ed., 1974, pp: 599; price: £ 2.80.

This book was first published in 1949 and has gone through six editions in the course of 25 years. The present edition has been enriched by inclusion of information obtained from surveys in many countries of Europe, the Middle East, the America, India, Ceylon and Burma undertaken by the first author on behalf of World Health Organization and Food and Agricultural Organization.

The presentation has been devided into 13 chapters dealing with meat animals, abattoirs, post mortem, inspection, tissues and organs of animals slaughtered for food uses, pathological and other lesions important from point of meat hygiene, inspection of poultry and game meat as rabbits and hares, preservation of meat and treatment and disposal of by-products.

The presentation includes an appendix which gives in the form of a statement the disease, source, affect and prevention of the common undesirable diseases encountered in the industry. The book is a good addition to libraries of institutions involved in work on the different aspects of the meat industry.

B. R. BALIGA

Proceedings of the International Symposium on Nitrite in Meat Products: Edited by B. Krol and B. J. Tinbergen, Centre for Agricultural Publishing and Documentation, Wageningen, The Netherlands, 1974, pp. 268; Price: DFL-45.

The use of small amounts of salt petre in the curing of meats is indispensable for the formation of the characteristic cured meat colour. The role of nitrite in develop-

ing cured meat colour was elucidated in 1890 and the mechanism was discussed by Haldane in 1901. Subsequent research workers discovered other characteristics of nitrite like inhibition of micro-organisms, particularly toxin forming anaerobes, clostridia and development of cured meat flavour.

One of the undesirable effects of use of nitrite in cured meat product, is the possibility of formation of nitrosamines. These nitrosamines pose a health hazard due to their carcinogenicity. When this drawback was recognised, debates started in many countries on the pros and cons of the use of nitrite in meat products in an international meeting at Zeist, the Netherlands in September 1973.

The discussion of the papers, about 25 in number, was grouped in four sessions, analytical, microbiological, technological and toxicological. At the end of each session, specific conclusion and recommendations were drawn up.

The analytical session discussed six papers on formation of cured pigment, determination of free and bound nitrite, low-molecular meat fraction active in nitrite reduction and determination of amines and oxides in food products. The main recommendations of this session were precise method for determination of nitrate, nitrite, volatile and non-volatile nitrosamines, precursors of nitrosamines and their combination in cured food products, by collaborative studies.

The microbiological session discussed six papers on microbiological effects of nitrite, inhibition of botulinum by curing salts in model systems in relation to stability and safety of cooked meats, minimum nitrite concentration for safety of cooked meats, Perigo effect and other inhibitions in cooked meat products. The important conclusions of this Session were the dependence of nitrite inhibition of clostridia on the salt concentration, temperature of storage, water activity, pH, type of food, etc., and the necessary dependence of the meat processing industry on nitrite for protection against clostridia and possibly staphylococci also. Any substitute for nitrite should be chosen with due consideration of its action on spore forming as well as non-sporing organisms.

The Session on Chemical and Technological Aspects of nitrites discussed loss of nitrite during storage of cooked meat product, influence of compounds other than nitrite on colour development, nitrite and flavour of cured meat, ascorbate and nitrosamine formation, low nitrate-nitrite and radiation sterlisation. The conclusions and recommendations of the Session were intense research to elucidate the mechanism of loss of nitrite binding to meat components, chemistry of cured meat flavour, reduction of nitrosamines in cured meats using additives like ascorbate and irradiation, particularly the balance between ascorbate and nitrite regarding nitrosamines and anti-botulinal effect.

The Toxicological Session dealt with papers on toxicity of nitrite and nitroso compounds, chemical carcinogens and formation of nitroso compounds. The conclusion and recommendations of this Session were that many N-nitroso compounds are powerful and versatile chemical carcinogens, trace quantities are present in foods like cured meat products and more could be formed in the gastrointestinal tract through interaction between nitrosating agents and nitrosotable amino compounds, epidemiological studies regarding health hazard for N-nitroso compounds, better and refined analytical methods of and advisability of reducing the amount of added as well as residual nitrite without affecting the protection against bacterial hazards, particularly botulins.

The overall conclusions of the Symposium was that nitrite is indispensable as an inhibitor of *Clostridium botulinum* in many meat products, plays a key role in colour and flavour development of cured meats. At the same time the presence of nitrosamines in some meat products is well established and refined analytical procedures, epidemiological studies and categorisation of products containing nitrite should be undertaken. So far the best available constituent to decrease the risk of nitrosamines is ascorbate because this does not impair the function of nitrites against clostridia.

Even though the compilation is predominantly of interest to research workers engaged in development of meat products and regulatory agencies, some of the information is of interest to research workers in other allied field like flavour, heat resistant bacteria encountered in processed products and mechanics of toxicological manifestation of chemicals formed during processing.

B. R. BALIGA

Seminar on 'Packaging of Marine Products' Organized by the Indian Institute of Packaging (Southern Regional Branch) held in Cochin on 4th March 1975.

The programme was participated by more than 45 delegates, representing various fish processing and packaging units, government and semi-government organisations, trade associations and research Institutes connected with marine products.

In his welcome address Shri A. I. George informed the delegates the various activities of the Institute and services rendered by the Institute to the industry. The industries in the Southern Region, was requested to make use of the facilities given by the Institute and take active participation in the programmes.

Shri K. Chidambaram, Director, Marine Products Export Development Authority, in his keynote address gave a brief account of the developments made by the marine industries in recent years. It was stated that the export of marine products was increased by more than 20 fold in ten year's time. There are many more marine products which are yet to be exploited. The main problem in exporting the marine product is packaging. The present export could be increased to a great extent by adopting improved techniques of processing and packaging.

After the general session, the technical session was conducted under the chairmanship of Shri K. Chidambaram, Member, Southern Regional Committee. Each technical session was presided by a technical expert from the field and 5 papers were presented which related to the different aspects of packaging of marine products.

The topics discussed in brief during the technical session are as follows:

Not much attention has been given to the improvements and standardisation of presently used frozen marine product carton. They are still packed in either 2 lb or 5 kg unit. It is suggested that greater economy could be achieved by standardising the type of cartons used for frozen fish.

Canning of marine products needs improvement for export market. At present only ordinary type of lacquered O.T. cans are used for fish canning. In export market they like to have these goods packed in special type of easy opening can which are yet to be manufactured in the country. Industries are of the opinion that if these containers are made available they will be able to export large quantity of canned fish. The can making industries are reluctant to go in to these containers because of the huge investment and uncertainity of demand estimate.

The packaging problem of dried fish for export is of different nature. The fish in the dried form has a very objectionable odour. If proper care is not taken regarding the moisture ingress, mould growth and other biochemical changes take place. Due to its bad odour these goods are not accepted by the shipping cargoes carrying tea, coffee and other spices. The technique used at present for drying is very crude. If better techniques are used for drying and packaging this highly protein rich food will get better price in the world market.

Not much improvement has been made in this country on transportation of goods under refrigerated conditions. Various types of shipping containers like plastic coated bamboo baskets, collapsible thermal insulated rigid containers, containers with refrigeration and cooling systems, vans with refrigerations and light weight bodies were discussed during the Seminar.

Following recommendations were made.

1. The paperboard carton used for frozen shrimps needs to be standardised. It was desired that a standard be developed for 5 kg unit. This standard will help the industry as guideline in selecting and specifying their packaging material.
2. The marine product industries were of the opinion that the export of canned marine products could be increased substantially, if proper type of containers are made available. In the foreign market the demand is for a special type of easy opening aluminium cans for canned products, which are yet to be manufactured in the country.
3. The packaging and processing techniques adopted for dried marine product needs improvement. The delegation was of the opinion that, if this protein rich product is hygeinically prepared by improved method of drying and packed in a protective and attractive container, it will fetch very good price in the export market. The M.P.E.D.A. was requested to study the problem in collobaration with IIP and Central Institute of Fisheries Technology, Cochin.
4. Much improvements are needed in the shipping container and transportation method adopted for marine products. The containers on which further study is needed are (*a*) plastic coated bamboo baskets, (*b*) collapsible thermal insulated containers, (*c*) containers with cooling systems, and (*d*) transportation system with better cooling systems.

The seminar was concluded with vote of thanks.

Report of the Seminar Programme Conducted by the Southern Regional Office of IIP on "Packaging of Food Products" at Madras.

The Southern Regional Office of the Indian Institute of Packaging organised a one day seminar programme on 'Packaging of Food Products' at Hotel Savera, Madras on 15th February 1975.

The programme was participated by more than fifty delegates from various food processing and packaging material supplier industry. In the programme various techniques adopted for food packaging were discussed.

In his welcome address Shri Sridharan explained the role of Indian Institute of Packaging in promoting the knowledge of packaging in the country. He gave a brief note on the present and future activities of the Institute and invited the industries in the Southern Region to enroll themselves as members of the Institute and take active participation in the Institute's activities. He also informed the delegates that the Institute is planning to conduct its "Indiastar Award 1975" function and a packing Exhibition in Madras later this year.

The keynote address for the programme was delivered by Shri P. V. Raju, Packaging Division, Central Food Technological Research Institute, Mysore. In his lecture he explained the status of food packaging in the country. He discussed the packaging problems involved in various types of food products like, oils and fats, fruits and vegetables, fish, meat and meat products, milk and milk products, bakery products, break fast cereals, confectionery, coffee and tea, milling products, foodgrains, breverages, spices, etc. He further explained that most of the food packaging materials and methods adopted in the country at present are based on the western countries research and development. We have to orient our research and development work suitably to suit our needs and demands.

The other items covered by the seminar programme are: Packaging of Canned Foods by Shri S. R. Shetty, Regional Officer, IIP, Packaging of Bread by Shri K. L. Radhakrishnan, General Manager, Modern Bakeries (India) Ltd., Packaging of Biscuits by Shri K. S. Kannan, Standards Manager, Britannia Biscuit Co. Ltd., Packaging of Confectionery by Shri H. R. S. Iyengar, Factory Manager, Parrys Confectionery Ltd., Packaging of Dairy Products by Shri Thomas Pathrose, Manager (Purchase), Tamil Nadu Dairy Development Corporation Ltd., Packaging of Dehydrated Foods by Dr H. B. N. Murthy, Marketing Manager, Travancore Rayons Ltd. A case study was presented by Shri S. R. Shetty, Regional Officer, IIP on evaluation of shelf life of a packed food.

During the group discussion several delegates brought out their problems connected with food packaging for export. All faculty members participated in the group discussion and helped the industries to solve their problems. The discussion comprised of selection of packaging materials, availability of materials and their limitations for packaging selection, correlation of packaging requirement of a product with packaging performance, etc.

Shri S. R. Shetty, Regional Officer, IIP, proposed the vote of thanks for the programme.

Faculty members answering the queries

Left to right:

1. Shri **S. R. Shetty**, *Regional Officer*, Indian Institute of Packaging, Madras.
2. Shri **K. S. Kannan**, *Standards Manager*, Britannia Biscuit Co. Ltd. Madras.
3. Dr **H. B. N. Murthy**, *Marketing Manager*, Travancore Rayons Ltd., Madras.
4. Shri **S. Sridharan**, *Production Executive*, Parrys Confectionery Ltd., Madras.
5. Shri **P. V. Raju**, *Scientist*, Central Food Technological Research Institute, Mysore.
6. Shri **K. L. Radhakrishnan**, *General Manager*, Modern Bakeries (India) Ltd., Madras.

ASSOCIATION NEWS

Minutes of the Annual General Body Meeting of the Association held at New Delhi on 27th April 1975.

The Annual General Body Meeting was held at Diocesan Community Centre, New Delhi under the Chairmanship of Sri K. C. Dé, Vice-president, Eastern Zone, AFST who was deputed on behalf of Dr T. N. Ramachandra Rao, President of the Association.

The Meeting opened with a welcome address by Mr K. C. Dé who expressed the hope that the members would have a good opportunity to examine the working of the Association during the last one year and also to propose any steps that would be required to further the interest of the Association.

The minutes of the last Annual General Body Meeting held at Mysore was then presented by the Hon. Exec. Secretary and the same was proposed for adoption by Mr Laljeet Singh and seconded by Mr. N. L. Jain. It was adopted unanimously.

The Annual Report for the year 1974 was then presented by the Secretary. During the discussion on the report, Dr Naik Kurade made a plea to assess the membership position of the Association with respect to the national register of Food Scientists and Technologists. The information on this may be collected for obtaining a realistic assessment of the role of the Association in collecting the Food Scientists and Technologists under the banner of one set up. It was also suggested by the members that for better recovery of arrears it is necessary to stop sending the Journal if any member fails to renew his membership after the posting of the first issue of every volume. After a lively discussion during which members expressed satisfaction on the working of the Association during the year, the report was proposed for adoption by Mr Y. K. Kapoor and seconded by Mr R. C. Bhutiani. It was adopted unanimously.

On behalf of the Hon. Treasurer, Hon. Exec. Secretary presented the statement of accounts for the year 1974 and the budget proposal for 1975. There was a proposal from Mr Y. K. Kapoor that it would be advisable to separate the accounts of the Journal and the Association so that the members will have an idea regarding the financial situation under these two different heads. It was explained by the Exec. Secretary that in view of the grant obtained from ICAR for bringing out the Journal it would be necessary for the Association to separate the accounts in future so as to satisfy the stipulations laid down by ICAR. The statement of accounts was proposed for adoption by Mr R. C. Bhutiani and seconded by Mr N. L. Jain which was adopted unanimously. The budget estimate was proposed for adoption by Mr Kailash Nath and seconded by Mr N. L. Jain. It was adopted unanimously.

The various resolutions forwarded by the Executive Council were taken up for discussion one by one.

a) The Ist resolution on Amendment to Article—V, Clause 1, B was accepted unanimously. The revised article will read as "The Immediate Past President and the Immediate Past Hon. Exec. Secretary".

b) The IInd resolution to taking steps for conduct examinations and awarding Diplomas was discussed in detail. The General Meeting felt that the idea is very commendable. The proposition was referred to Executive Council for examining the same in detail and come up with recommendations in the next General Body Meeting.

c) The IIIrd and IVth resolutions coming from Hyderabad and Bangalore Chapter respectively regarding the shifting of Southern Zone Office were rejected and it was decided to examine these proposals in the next General Body Meeting.

d) The Vth resolution proposed by the Executive Council for instituting Subrahmanian Award was accepted unanimously.

e) The VIth resolution on holding of elections for the office of Vice-president of Head-quarters whenever he/she transferred was rejected by the General Body.

f) The last resolution on changing the emblem of the Association was discussed in great length. Since there were different opinions the resolution was put to vote and rejected by majority vote. The meeting decided to authorise the next Executive Council to expeditiously examine the dsirability of having a new emblem and arrive at a better design in consultation with some of the established designers.

Other suggestions put forward for consideration by E.C. included.

1. Mr Y. K. Kapoor, Secretary of All India Food Preservers' Association declared in the meeting that the Association would be willing to contribute substantially for setting up an Institute which would be able to further the interests of the food industry by the process of education and training. This may be taken up by the next E.C. for further action.

2. Dr S. P. Manjrekar, Chairman, Technical Committee of AIFPA proposed that AFST might consider inviting a representative from AIFPA as a permanent invitee in their deliberations. It was pointed out that AIFPA has already made a decision to associate AFST with their activities. The concensus was that the above proposition is worth considering.

3. Mr Kailash Nath of M/s. Harnarian and Gopinath, New Delhi announced that he is considering instituting an award for outstanding work in the area of traditional food products. The next E.C. may finalise this in

consultation with Mr Kailash Nath. Similarly Dr A. G. Naik Kurade of M/s. Suman Food Consultants, New Delhi also announced his intention to set up an Award details of which would be forwarded to the next E.C. for further processing. The Meeting placed on record the appreciation by members on the above proposition and Secretary expressed his thanks on behalf of the Association.

Gardners' Award for the year 1973 was then presented by Dr P. K. Kymal, Executive Director, Food and Nutrition Board, New Delhi to the recipiants which was accepted on behalf of the Authors by Dr V. H. Potty.

Mr K. C. Dé, Chairman of the meeting then introduced the President-elect who is to become the President for the year 1975. After highlighting the achievements of Dr Kymal, he was inducted as the New President for 1975. Dr Kymal in his remarks indicated that he has an effective programme to further causes of the association and he appealed for cooperation and collaboration from all the members of the Association. He also appreciated the good work put in by the Executive Council and of the Office-bearers of the previous year. President Dr Kymal then announced the results of the election held for various AFST Offices.

A vote of thanks was proposed by Mr P. N. Narang.

V. H. Potty

III National Symposium on Refrigation and Air-Conditioning

In accordance with the decision taken to hold Symposium every year on the subject of Refrigeration and Air-Conditioning in different parts of the country, to focus the attention of all concerned on this vital subject, the 3rd National Symposium on Refrigeration and Air-Conditioning was organised by the CFTRI, Mysore for 3 days from 18th to 21st July 1974. The Symposium was sponsored jointly by the CFTRI, Mysore, Ministry of Defence, Government of India, All India Air-Conditioning and Refrigeration Association, Association of Food Scientists and Technologists (India) and the Institution of Engineers, Mysore City Sub-Centre, Mysore. It was attended by nearly 140 delegates consisting of research scientists, design engineers, manufacturers of machinery and equipment, management executives and government officials.

The Symposium was inagurated on 18th July 1974 by Dr. A Ramachandran, Secretary, Department of Science and Technology, Govt. of India and was presided over by Sri D. J. Balaraj, Development Commissioner, Government of Karnataka. The following is a summary of the Inagural address delivered by Dr A. Ramachandran. The general theme of his talk was "Heat and Mass Transfer" which is fundamental to refrigeration and air-conditioning.

Dr A. Ramachandran's Address

Heat and mass transfer in food products, whether they are of vegetable or animal origin, is a complicated problem, because, consideration has to be given to quality retention during processing and this imposes severe restrictions on processing operations. In order to study any problem of heat or mass transfer in food products, it is necessary to have complete data relating to their thermo-physical and moisture transport properties under varying conditions of temperature, humidity, physical state, source from which the material is obtained and processing conditions. In view of the large number of variable parameters involved, it is best to determine these properties experimentally in each case in a systematic fashion and tabulate them for later use. This is a very important step in the economy of the processes and in controlling the quality of the products.

Cooling of food products to retard their deterioration during storage and freezing to achieve increased storage life is a well known process. Determination of the cooling loads and time-temperature characteristics is important to exercise control over the quality of products and economy of processes. Moisture migration and the controlling effect of the surface moisture film have a profound influence on the processes. While studies on cooling of specific food products have been made in various laboratories, generalised studies depicting the influence of various parameters on cooling processes are scarce. Such studies if made, will help in predicting the performance of different products during the cooling process, under varying conditions.

Similarly, while extensive studies on freezing of various products to understand the basic mechanism of freezing have been made, there is still a necessity to make generalised studies on mathematical models to predict freezing characteristics of anamolous shapes. Also studies on cryogenic freezing with LN2 and LF22 sprays with a view to evolve the most economic cooling system and with minimum product damage are needed.

The other process involving both heat and mass transfer relating to food products is dehydration. The important consideration in dehydration is once again economy and quality retention. In most cases, air is used as the heat and mass transfer medium for solid foods. For food materials in liquid form, drum drying, spray drying, foam mat drying and vacuum puff drying methods are used. Vacuum freeze drying is gaining popularity of late, because of the high quality product it yields though it is still considered as a costly process.

Food products have to be dehydrated under "low intensity" and "high moisture" conditions for quality retention, and under these conditions, rate of moisture diffusion within the body and surface evaporation are controlling factors. 'Spiked plate freeze drying' and 'Accelerated freeze drying' fall into this category. A recent trend in these techniques is to operate at not to high a vacuum and introduce gases like nitrogen, helium, etc. This brings in the concept of convective heat transfer in addition to conduction and radiation effects in freeze drying. Detailed study of these heat transfer systems is needed to predict the freeze drying characteristics under these conditions. Also reduction in particle size of the product into pebbles or granules helps in reducing processing time. A technique recently being tried out is 'spray freeze drying' wherein the advantages of both spray drying and freeze drying are incorporated. Liquid is sprayed into an evacuated chamber and the spray deposits on the drier wall, where the heat of sublimation is supplied. This seems to be a promising method and deserves greater attention and detailed study.

Concluding Dr Ramachandran dealt on the intensive studies made on the economic conversion of solar energy into useful 'heat' or 'cold'. Applications of solar energy in heating are water heaters, boilers, air-heaters and drying of foodstuffs. Its application in 'cold' is in harnessing the energy for refrigeration and air-conditioning, particularly for absorption refrigeration. Other areas are in evaporation of liquid foods, blanching, cooking and peeling; creation of vacuum, etc. All these make very interesting study, he said.

Sri D. J. Balaraj's Address

The following is a summary of the Presidential Address delivered by Sri D. J. Balaraj, Development Commissioner, Government of Karnataka.

Broadly, refrigeration and air-conditioning impinge on both agricultural and industrial policies and programmes. While on the agricultural sector, it helps in preserving perishable materials over longer periods without deterioration in quality and elimination of waste, on the industrial sector, it helps in careful husbanding of our resources and proper utilisation of installed capacity in various spheres. For lack of proper storage and handling, nearly 20–25% of our perishable food products, goes to waste at present. The refrigeration industry can play a vital role in minimising this waste to a large extent.

While enumerating the extent of perishable food products produced in Karnataka State and efforts being made by Government and other agencies in providing, processing and storage facilities for such products, Sri Balaraj stated that the World Bank has sanctioned a grant of Rs. 50 crores for animal husbandry development in the State over a 7 year period, and indicated that this calls for a large outlay on refrigeration and air-conditioning plants for various processes involved such as chilling and processing centres for milk, storing and freezing of meat, fish and other products and so on. He also emphasized the need for maintaining a cold chain for the transportation of such stored and processed products, over long distances and suitable marketing facilities at different centres.

Cold storage of food is only one important application of refrigeration, he said. There are many other useful applications such as cooling of large masses of concrete in dam construction, providing chilled water for industrial applications, ice plants for the fish industry, air-conditioning of hospitals, pharmaceutical and chemical industries, aircraft optical goods, production centres, instrument rooms and so on, which are essential necessities and can by no means be termed as "Luxuries".

In spite of such a versatile application of refrigeration and air-conditioning in various fields, one finds that the installed capacity of the machinery industry is very much under-utilised. Consequently the prices of machinery are rather high. In addition one hears the complaint that excise duty and sales tax on such machinery and equipment is very high as these are termed as luxury items. While there may be some justification to the demand for reduction in duties, the better way is to utilise the installed capacity fully and the equipment used to cater to the masses (instead of individuals who come within the top 10% of income) by installing large cold stores even in rural areas to preserve the available food materials.

Concluding Mr Balaraj said that symposia like this held in centres like CFTRI where a large amount of work has been done regarding preservation of perishable food materials by refrigeration, would help bring together men from industry, trade, R & D organisations for exchange of useful information and understanding each other's problems to bring the results of research within the reach of the common man. He also emphasized the need for training programmes for engineers and technicians in large numbers for better maintenance of the equipment for their optimum and economic utilisation, since the industry is capital intensive.

Technical Sessions

The Symposium met in 5 Technical Sessions and 40 papers were presented. Latest researches and techniques, design and development of machinery, etc., in the field of refrigeration and air-conditioning were discussed. It brought into focus the problems of the industry in areas where developmental work are needed. The recommendations of the Symposium are given below:

Technical Session I: Cold Storage of Perishable Products

Chairman: Shri N. V. Baxi, General Manager, M/s. Voltas Ltd., Bombay.

Recommendations

Research on the following areas should be intensified and accelerated with a time-bound programme so that it could be put to beneficial use on a commercial scale in view of the critical food shortage in the country.

1) Using irradiation and refrigeration on certain perishable products including their cost structure.
2) Commercial scale freeze drying of perishable commodities with special reference to fruit juices and evaluation of comparative cost vis-a-vis other techniques.
3) Feasibility of using individual/instant quick-freezing methods for sea foods, ready-to-serve foods, fruit and vegetable products with liquid nitrogen and different refrigerants.
4) Intensification of efforts for extending the storage life, prevention of low temperature break-down and maintaining the organoleptic qualities of different tropical fruits with particular reference to mangoes.
5) Special attention to be given for establishing adequate refrigeration facilities in abattoirs and for the preservation of subsidiary products with special reference to pancreas (required for the manufacture of insulin).

Technical Session II: Heat Transfer & Thermodynamics

Chairman: Brig. S. C. L. Mullick, Defence Ministry, Government of India, New Delhi.

Recommendations

1) Further studies and investigations should be carried out to standardise the techniques of heat transfer through heat pipe in cryogenic fields and pulse tube refrigeration.
2) Computations regarding the losses in refrigeration should be thoroughly investigated in view of the energy crisis.
3) Further experiments should be continued on freeze drying to understand the basic mechanisms and to develop various thermodynamic equations in view of its urgent use for defence purposes.
4) Possibilities of reducing the cost of refrigeration in freeze drying should be investigated.
5) Experimental investigations should be strengthened on studies of vapour-liquid equilibria for refrigerant mixtures.
6) Various parameters and data required for building enthalpy concentration charts should be studied.
7) Studies on heat and mass transfer for various tropical food products should be strenghtened.
8) Computer methods should be evolved for standardization and computation of various thermodynamic properties in S. I. units.

Technical Session III: Design and Construction of Cold Storages

Chairman: Prof. C. P. Arora, Indian Institute of Technology, New Delhi.

Recommendations

1) Standard and economic designs for cold stores in various sizes and for various products should be prepared by R & D organisations for the benefit of industry and consumers.
2) Studies should be made to make multiple utilization of cold stores possible for different products at one and the same time, varying with seasons, in different regions of the country.
3) Cheap indigenous insulations such as paddy husk should be studied along with the vapour barriers suitable for use with them. For this, it is necessary to establish test facilities at a central place for finding out the permeability of moisture through insulations as well as through vapour barriers.
4) Design of doors for frozen food stores may be further studied and perfected.
5) It may be noted that the requirement of a vapour barrier can be waived in jacketed stores since the insulation is generally at a higher temperature than the dew point temperature of the air. Accordingly, cheaper quality insulations can be used in such cold stores.
6) Existence of one refrigerated sea water plant was reported for fish preservation. Many more such plants and plants of other designs may be tried. When compared to the cost of freezing on board, this method would save the cost of labour required for the pre-cleaning and processing of fish, and would also avoid the thawing that would occur before final processing and packing on shore. The method may also prove useful for storage of fish in processing plants on the shore.
7) R & D efforts on controlled atmosphere storage should be intensified.

Technical Session IV: Machinery and Equipment Used in Cold/and Freezer Storage

Chairman: Shri Manmohan Singh, Managing Director, M/s. Frick India Ltd., New Delhi.

Recommendations

1) Present R & D activities of various universities and laboratories in the field of refrigeration are of vital importance to the industry. There should be close collaboration and exchange of ideas between research and industry for better utilisation of R & D efforts.
2) The industry should be requested to pose their problems to IIR which in turn may sponsor R & D projects to be taken up by the research organisations.
3) The computer simulation programme for DX-chillers as outlined could be beneficially utilised by the manufacturers of machinery in order to effect saving of scarce raw materials.
4) Suitable similar programmes for optimisation of investment may be initiated in research centres for other refrigeration machinery.
5) Of late, liquid nitrogen freezing is gaining popularity in view of its inherent advantages. Industry should sponsor research and developmental activity in this field.
6) Refrigerating machinery for cold storages and freezing plants suffer from a very high rate of excise duty due to an erroneous impression that it caters to the affluent section of society. In order that the machinery could be effectively used in the field of preservation of foods, by lowering its costs, there is need for drastic reduction of central excise duty.

Technical Session V: Air-conditioning

Chairman: Shri Ram D. Malani, Executive Director, M/s. Blue Star Ltd., Bombay.

Recommendations

Unfortunately in this country, air-conditioning has acquired a mistaken connotation. Few people realise that air-conditioning is indispensable in a number of modern industries like pharmaceuticals, chemicals, fertilisers and precision engineering. Similarly air-conditioning is vital for operation theatres, telephone exchanges and radar systems now being extensively used by defence services. It will therefore be wrong to describe the air-conditoning industry as a luxury industry. Almost 80% of the output of this industry is used for industrial application and it is therefore vital that this industry should be allowed reasonable opportunities for growth.

In the field of comfort air-conditioning, it is possible, within certain limitations, to use the evaporative cooling systems. It must however be understood that evaporative cooling will be effective only in dry climates. Even here, evaporative cooling would work only for 2 to 3 months a year (from end of March to end of May). With the onset of monsoon there is a rise in the relative humidity which makes evaporative cooling ineffective. Similarly, evaporative cooling cannot function in applications where there is concentrated human occupancy, for e.g. cinema theatres, auditoria, lecture halls, etc.

Work may be undertaken to precisely determine the effective inside temperature range applicable to Indian conditions for evaporative cooling.

General Recommendations

1) The Symposium recommends that the NCIIR and the AIARA should establish a liaison with all institutions doing work in the field of refrigeration and air-conditioning and bridge the existing communication gap between institutions and institution on one hand and industry on the other.
2) It is recommended that the NCIIR should take appropriate follow up action regarding the recommendations made by the Symposium and report the action taken at the time of the next Symposium.
3) It is recommended that the Proceedings of the Symposium be brought out as a separate publication after editing of all the papers presented along with the recommendations.
4) It is also recommended that the future organisers of the Symposium devise such means as to reduce the number of papers to be presented at the Symposium and give more time for discussions and as far as possible supply pre-prints of papers.
5) It is recalled that in the 1st National Symposium held at Durgapur in 1972, a recommendation had been made that action should be taken to bring out a Refrigeration Data Book for use in India compiling all available data in the field on the model of the ASHRAE Handbook and Guide. It is also recalled that the ISI had undertaken to bring out this compilation. It is recommended that the NCIIR may take suitable action to bring out this publication in consultation with the ISI.
6) It is recommended that short term courses on specific topics in the field of refrigeration and air-conditioning, cold and freezer storage should be held periodically at institutions where such facilities exist like the CFTRI, CMERI, BARC, etc., and NCIIR should take a lead in organising these courses.
7) It is recommended that to encourage good quality papers being presented, the NCIIR should institute a prize for the best technical paper prepared and presented at such Symposia.

8) It is recommended that in future only metric system of units should be adopted in all papers presented at the national symposia.

Forty papers were presented at the Symposium; the full text of these papers will be published shortly.

AFST, Bangalore Chapter

The Bangalore Chapter of the Association of Food Scientists and Technologists met on 18-3-1975 along with the Institute of Standards Engineers, Bangalore Section. Shri C. K. Ramanathan, Manager, Brooke Bond addressed the joint meeting on Standards for Coffee. Dr N. C. Chokkanna, Retd Director of the Central Coffee Research Station presided.

Shri Ramanathan described the various factors that have been taken into consideration in fixing the standards for coffee. The coffee industry is one of the major foreign exchange earning industries. The foreign market has to be satisfied and it is in this connection that chicory has been permitted as an additive to coffee for certain grades. But for the availability of chicory and its use along with coffee, it would not have been possible for the Indian production to meet both the Indian market and the foreign market. Dr. Chokkanna in his remarks said that the work on the quality of coffee was taken up by the Agricultural Department of the Mysore State long ago and was continued later as a scheme for improvement of the quality of coffee with factors like aroma, body, acidity, etc. Cup tasters were employed both in England and in India and their assessment checked statistically. Other factors like the environment, the soil, manure, and variety of the bush were also studied. Thus the pioneering efforts in assessing quality of coffee and its standardisation were carried out at Bangalore in the Mysore Agricultural Department. There was a lively discussion on the use of chicory in coffee, opinions ranging from its necessity, to it being called an adulterant. The talk ended with a vote of thanks by Shri Subramanian of Indian Standards Institute, Bangalore.

New Members

Mr. Hiranmay Gangopadhyay, Department of Food Technology and Biochemical Engineering, Jadhavpur University, Calcutta-700 032.

Mr. Bhudeb Gupta, Chemist-in-charge, Reckitt & Colman India Ltd., P.B. No. 9002, Calcutta-16.

Mr. Byomkesh Nandy, Chemist cum Manager, Alpha Foods & Chemicals (P.) Ltd., Tollygaunj, Calcutta-53.

Mr. Sudev Kuman Mandal, New Block Hostel, Jadhavpur University, Calcutta-700032.

Mr. Shyamal Kumar Bhattacharya, Biochmical Engineering and Food Technology Department, Jadhavpur University, Calcutta-32.

Mr. Madhu Sudan Karak, New Block Hostel, Jadhavpur University, Calcutta-700032.

Mr. H. K. Vasuki, Production-in-charge, Aurofood Pvt. Ltd., Auroville P.O., Tamilnadu.

Dr. A. S. Aiyar, Biochemistry and Food Technology Division, Bhabha Atomic Research Centre, Trombay, Bombay-85.

Mr. A. S. Ghanekar, Biochemistry and Food Technology Division, Bhabha Atomic Research Centre, Trombay, Bombay.

Mrs. Lalitha G. Govekar, Biochemistry and Food Technology Division, Bhabha Atomic Research Centre, Trombay, Bombay-400 085.

Mr. Macchindra Tukaram Jamave, Biochemistry and Food Technology Division, Bhabha Atomic Research Centre, Trombay, Bombay-400 085.

Mr. Madhusudhan R. Joshi, Biochemistry and Food Technology Division, Bhabha Atomic Research Centre, Trombay, Bombay-400 085.

Mr. Madhusudhan Y. Kamat, 28, Vincent Square, Street No. 2, Dadar, Bombay-400014.

Dr. M. S. Netravali, Biochemistry and Food Technology Division, Bhabha Atomic Research Centre, Trombay, Bombay-400 085.

Mr. B. Y. Krishnoji Rao, Biochemistry and Food Technology Division, Bhabha Atomic Research Centre, Bombay-400 085.

Mr. U. Y. Rege, Manager, Raptokos Brett and Co. Ltd., Worli, Bombay-400 025.

Mr. Shridhar Krishna Sathe, Joshi Building, Hanuman Road, Vile-parle Bombay-57, AS.

Mr. B. L. Satyanarayana, Department of Chemical Technology, Foods Section, Matunga, Bombay-400 019.

Mr. M. Jayaprakasha Rao, Production Manager, M/s. Janfa Fruit Products, Sagar, Shimoga, Karnataka.

Mr. Parikshit Roy, 157, Sarat Bose Road, Calcutta-26, West Bengal.

Mr. Sandup Norden, Fruit Preservation Factory, Government of Sikkim, P.O. Singtam, Sikkim.

Dr. U. M. Ingle, Project Officer, Faculty of Agri. Technology, Marthwada Agricultural University, Parbhani.

Dr. D. K. Salunke, Vice-chancellor, Marathwada Agricultural University, Parbhani-431401.

Dr. M. R. Salunke, Botany Department, Marathwada Agricultural University, Parbhani.

Dr. H. Onkarayya, Training Centre, C.F.T.R.I., Mysore-13.

Mr. D. N. Gandhi, Dairy Bacteriology Division, National Dairy Research Institute, Karnal, Haryana.

Mr. N. B. Hulamani, Senior Asst. Manager (P & S) Karnataka State Agro Corn Products Limited, Belgaum, Karnataka.

Mr. K. Manohar, Chemist, M/s. Chennai Bottling Co. P. Ltd., Arumbakkam, Madras-29.

Mr. Chandra Mohan Bhargawa, 16, 3rd Cross Street, Shenoyanagar, Madras-30.

Mr. Birendra Bahadur, Asst. Technical Director, Export Inspection Council, Calcutta-700001.

Mr. Dilip Kumar Adhikary, Food Technology & Biochemical Engineering, Jadhavpur University, Calcutta-700032.

Mr. Rajinder K. Gupta, Partner, National Fruit Products, Salem Tabri, Ludhiana.

Mr. Dharam Pal Bansal, National Fruit Products, Salem Tabri, Ludhiana.

Mr. M. Ramesh Babu, PDD Discipline, CFTRI, Mysore-570013.

Mr. Mohan P. Asrani, Technical Adviser, Asrani Enterprises, Bangalore-53.

Mr. S. P. Kamat, Technical Advsier, Western Goa Confectionery, Bainginim, Old-Goa P.O., Goa.

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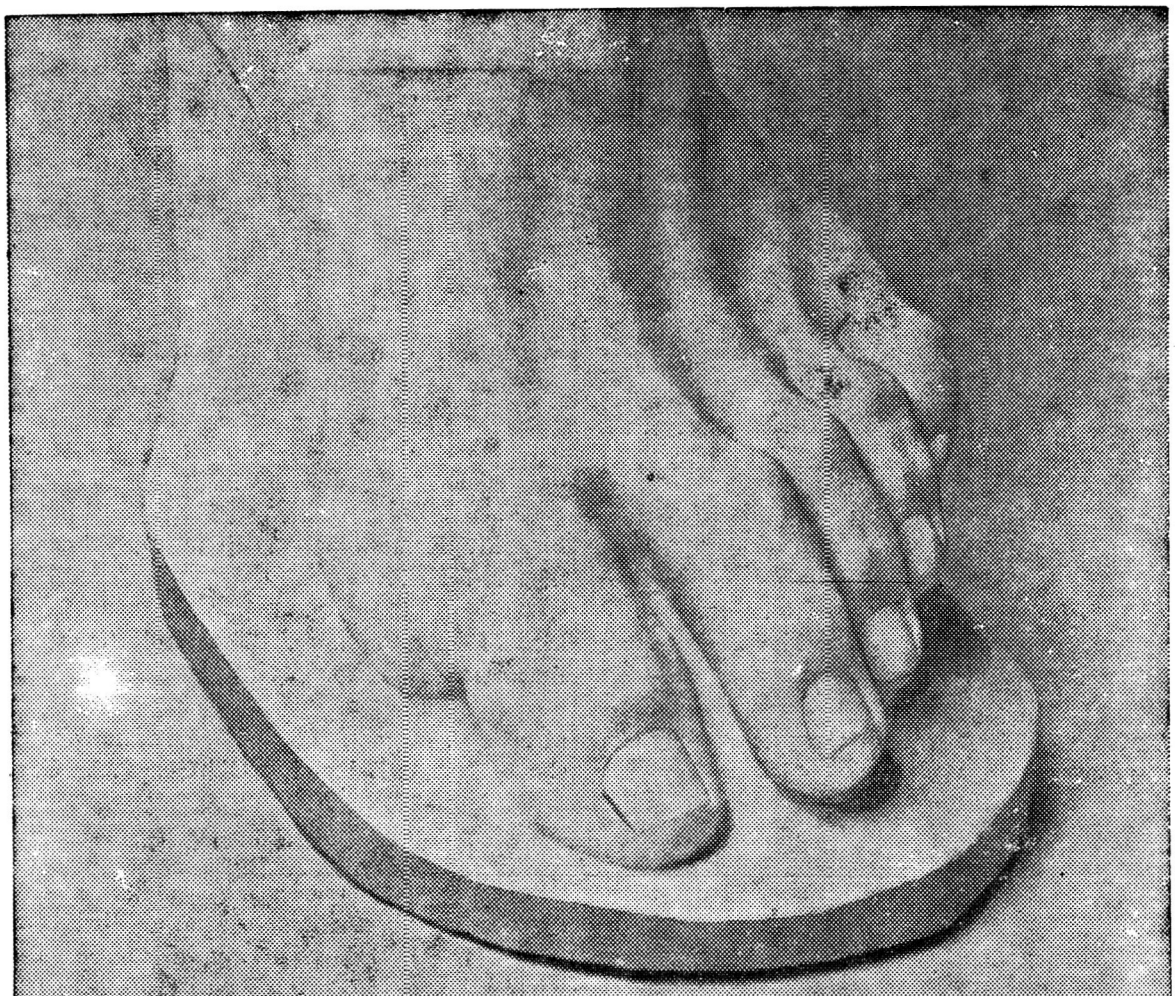

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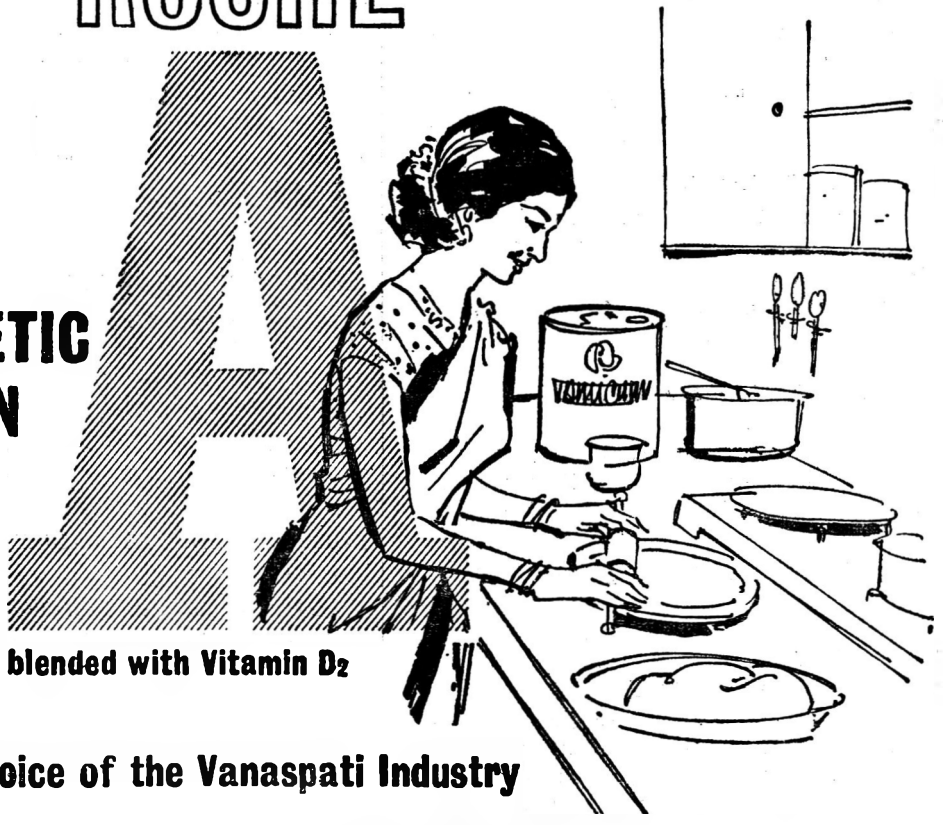

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iii) Quality control and standards (8 papers)
iv) Spice flavours and products (5 papers)
v) Marketing of spice and spice products (4 papers)
vi) Future needs of spice industry (7 papers)

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